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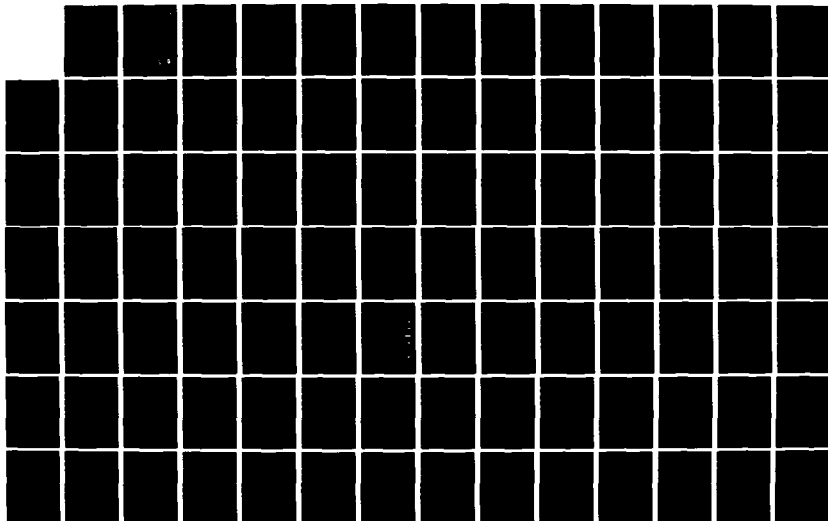
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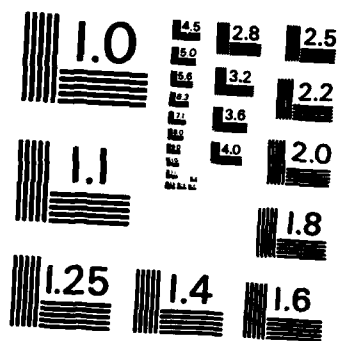
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Technical Report E06549-17
Contract No. N00039-84-C-0070

IITRI

AD-A159 184

COMPILATION OF 1984 ANNUAL REPORTS
OF THE NAVY ELF COMMUNICATIONS SYSTEM
ECOLOGICAL MONITORING PROGRAM

Volume 1 of 2 Volumes: TABS A-E

June 1985

Prepared for:

Space and Naval Warfare Systems Command
Communications Systems Project Office
Washington, D.C. 20363

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Printed in the United States of America

This report is available from:

National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161

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SECURITY CLASSIFICATION OF THIS PAGE

AD-A159184

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution unlimited		
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA					
4. PERFORMING ORGANIZATION REPORT NUMBER(S) E06549-17			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION IIT Research Institute		6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) Chicago, Illinois 60616			7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Space and Naval Warfare Systems Command		8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00039-84-C-0070		
8c. ADDRESS (City, State, and ZIP Code) Washington, D.C. 20363			10. SOURCE OF FUNDING NUMBERS		
			PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO. Task H
			WORK UNIT ACCESSION NO.		
11. TITLE (Include Security Classification) Compilation of 1984 Annual Reports of the Navy ELF Communications System Ecological Monitoring Program (Volume 1 of 2 Volumes) (Unclassified)					
12. PERSONAL AUTHOR(S) See Block 19					
13a. TYPE OF REPORT Annual Progress Report		13b. TIME COVERED FROM Jan 1984 to Dec 1984		14. DATE OF REPORT (Year, Month, Day) June July 1984	
				15. PAGE COUNT 548	
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
			Ecology		
			Electromagnetic Effects		
			Environmental Biology		
			Extremely Low Frequency		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) A long-term program of monitoring for possible ELF electromagnetic influences on ecosystems in northwestern Wisconsin and the Upper Peninsula of Michigan is being conducted. Selection of study sites, monitoring protocols, and analytical methods were initiated in 1982. These activities, as well as data collection, were continued during 1983 and 1984. Progress is described for studying the terrestrial, aquatic, and wetland ecosystems for the 11 projects comprising the Ecological Monitoring Program. See reverse for report titles and authors.					
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL			22b. TELEPHONE (include Area Code)		22c. OFFICE SYMBOL

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SECURITY CLASSIFICATION OF THIS PAGE

19. Abstract (continued)

Report titles and authors:

Herbaceous Plant Cover and Tree Studies

Anderson, M.; Bruhn, J.; Cattelino, P.; Jurgensen, M.; Lenz, G. W.;
Liechty, H. O.; Mroz, G.; Reed, E. J.; Schultz, J.; Trettin, C.

Litter Decomposition and Microflora

Bagley, S.; Bruhn, J.; Jurgensen, M.

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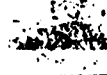
Goodman, E. M.; Greenebaum, B.

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Soil and Litter Arthropoda and Earthworm Studies

Snider, R. J.; Snider, R. M.

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DTIC TAB	<input type="checkbox"/>
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FOREWORD

This document is the third compilation of Annual Reports on the Extremely Low Frequency (ELF) Communications System Ecological Monitoring Program initially authorized under Naval Electronic Systems Command Contract N00039-81-C-0357 to IIT Research Institute (IITRI). The studies in this Program are being continued under Space and Naval Warfare Systems Command Contract N00039-84-C-0070. IITRI provides engineering support and coordinates the efforts of investigators in 11 studies, all of which are being conducted under sub-contract arrangements (E06549-84-C-001/011) between IITRI and the study teams.

The purpose of the Ecological Monitoring Program is to determine whether electromagnetic fields produced by the Navy's ELF Communications System will affect resident biota or their ecological relationships. Biological aspects of 16 general types of organisms and ecological aspects of three ecosystems are being monitored in Wisconsin and Michigan.

The originally proposed study objectives, monitoring protocols, and analytical techniques were presented in the 1982 compilation of annual reports. Subsequent changes and study progress are documented in the 1983 compilation and in this one. Major activities of the program initiated in 1983 and continued during 1984 are the collection of information to validate assumptions made in proposals, the identification and characterization of critical study aspects, and the selection of study sites.

Commencing in 1983, each annual report has been reviewed by four scientific peers. Two of the four are selected by the reporting investigator; the other two are selected by IITRI. Critiques are supplied to the authors for their consideration in finalizing their annual reports and in planning the next field season.

This compilation was printed from original copies of each investigator's report for 1984 without change or editing by either IITRI or the Space and Naval Warfare Systems Command.

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**ELF ECOLOGICAL MONITORING PROGRAM
INDEX OF 1984 ANNUAL REPORTS**

- A. Herbaceous Plant Cover and Tree Studies
Michigan Technological University
Anderson, M.; Bruhn, J.; Cattelino, P.; Jurgensen, M.; Lenz, G. W.;
Liechty, H. O.; Mroz, G.; Reed, E. J.; Schultz, J.; Trettin, C.
- B. Litter Decomposition and Microflora
Michigan Technological University
Bagley, S.; Bruhn, J.; Jurgensen, M.
- C. The Effects of Exposing the Slime Mold Physarum polycephalum
to Electromagnetic Fields
University of Wisconsin-Parkside
Goodman, E. M.; Greenebaum, B.
- D. Soil Amoeba
Michigan State University
Band, R. N.
- E. Soil and Litter Arthropoda and Earthworm Studies
Michigan State University
Snider, R. J.; Snider, R. M.
- F. Biological Studies on Pollinating Insects: Megachilid Bees
Michigan State University
Fischer, R. L.
- G. Small Vertebrates: Small Mammals and Nesting Birds
Michigan State University
Beaver, D. L.; Asher, J. H.; Hill, R. W.
- H. Aquatic Ecosystems
Michigan State University
Burton, T. M.; Merritt, R. W.; Stout, R. J.; Taylor, W. W.; Oemke, M. P.;
O'Malley, M.; Whelan, G.; Cornelius, D.; Gesl, D.; Webb, K.
- I. Wetland Studies
University of Wisconsin-Milwaukee
Stearns, F.; Guntenspergen, G.; Keough, J.; Wikum, D.
- J. Field Studies of Effects of ELF on Migrating Birds
Illinois Natural History Survey
Larkin, R. P.
- K. Bird Species and Communities
University of Minnesota-Duluth
Niemi, G. J.; Hanowski, J. M.

ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM:

HERBACEOUS PLANT COVER AND TREE STUDIES

The Michigan Study Site

Tasks 5.13/5.14

ANNUAL REPORT 1984

SUBCONTRACT NUMBER: E06549-84-C-001,002

MICHIGAN TECHNOLOGICAL UNIVERSITY

HOUGHTON, MICHIGAN

ELF COMMUNICATIONS SYSTEM ECOLOGICAL MONITORING PROGRAM:
HERBACEOUS PLANT COVER AND TREE STUDIES

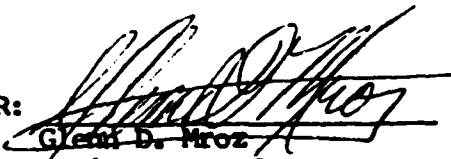
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ANNUAL REPORT 1984

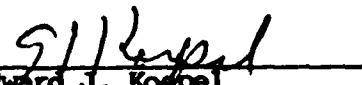
SUBCONTRACT NUMBER: E06549-84-C-001,002

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Table of Contents

	<u>Page</u>
Introduction	1
Element 1: Plot Selection	4
Element 2: Development, Installation and Operation of the Ambient Monitoring System.	29
Element 3: Tree Productivity.	32
Element 4: Phenophase Description and Documentation	60
Element 5: Herbaceous Vegetation Cover and Growth	70
Element 6: Mycorrhizal Fungi Collection	75
Element 7: Mycorrhiza Characterization and Root Growth.	88
Element 8: Litter Production	96
Element 9: Data Management.	103
LITERATURE CITED	106
APPENDIX A: Summary report of vandalism at the trees and herbaceous plant cover control site	109
APPENDIX B: Summary of electromagnetic field measurements	133
APPENDIX C: Equations used in determining whole tree biomass.	139
APPENDIX D: Soil profile description and compositional analysis of the soil occurring on the control study area.	145
APPENDIX E: Analysis of the composite soil samples from the control site.	151
APPENDIX F: Configuration of the ambient monitoring platforms	159
APPENDIX G: NESS data retrieval procedure	165
APPENDIX H: Seasonal diameter growth of each species on the antenna and control sites	169
APPENDIX I: Timber volume figures for research plots.	179
APPENDIX J: Average values for seedling measurements by site and date-first growing season	183
APPENDIX K: Structure of SIR DBMS and description of records associated with the Trees and Herbaceous Plants Task.	187

INTRODUCTION

Since forest vegetation is dominant on the proposed ELF communications antenna area, it is essential to include it in an ecological monitoring program. However, there are several other considerations which justify their study. Trees and herbaceous plants having both above and below ground biomass will be more closely coupled to the ELF field than those organisms solely in the air or on the soil surface (Anonymous, 1977). Trees differ from herbaceous plants in that they are more deeply rooted and are longer lived, while herbaceous plants have been found to be more sensitive to site disturbance than trees (National Research Council, 1977). However, trees offer the unique opportunity to evaluate effects of the ELF electromagnetic field on the same individuals over a much longer period of time while also evaluating changes in stand dynamics. These considerations would be of paramount importance in assessing the significance of ELF field effects occurring at the organismal level.

A secondary consideration is that forest vegetation also exerts strong influence on other organisms within the ecosystem, both above and below ground. These effects include modifying microclimate, exerting influence on soil organisms, particularly in the rhizosphere, and by influencing soil development and fertility through nutrient cycling. By studying the effects of ELF fields on individual plants and plants in the ecosystem, information gained will also be useful to investigators studying other ecological relationships in the ELF environmental monitoring program.

While there are many measures of tree response to any given stand treatment, only a few are generally needed to quantify that response and test its significance. These measurements must be chosen on the basis of highest sensitivity, especially in the case of ELF field effects, since previous work has indicated these may be extremely subtle (National Research Council, 1977). In addition, the measurement must be practical so it can be accomplished as part of a field study outlined for the overall ELF ecological monitoring program. Based on these constraints, the Tree and Herbaceous Plant study has been divided into the following separate elements: (1) plot selection, (2) development, installation, and operation of the ambient monitoring system, (3) tree productivity, (4) phenophase description and documentation, (5) herbaceous vegetation cover and growth, (6) mycorrhizal fungi collection, (7) mycorrhizal characterization and root growth, (8) litter production, (9) computer program development and analysis.

The broad objectives of the study remain: 1) to investigate and characterize the growth of trees and herbaceous plants on selected plots within the ELF antenna area prior to operation of the antenna and 2) use this baseline data to evaluate the possible effects of ELF electromagnetic fields on plant growth.

The major emphasis in this years work has been to implement those studies outlined as departures from our original research program. The most significant of these changes included the planting of red pine (Pinus resinosa) for the study of possible field effects on ectomycorrhizal fungi and on the growth of rapidly growing seedlings. To this end, permission was granted by the Navy, and Michigan DNR to clear and plant red pine on approximately 2 hectares at each of three sites. Although seedlings were

planted somewhat late in the Spring because of delays in plot selection and clearing, seedling survival has been good. All phases of our study were operative until the occurrence of extensive vandalism late in the year. Because this damage occurred after the growing season, it had little effect on the results presented in this report, but is of much greater concern as we now plan for the next field season. The element most impacted was the herbaceous cover and growth study. We are currently concerned with restoration of the previous herbaceous plant study plots or possibly changing the research approach. For a full account of vandalism, see Appendix A.

ELEMENT 1. Plot Selection

Detection of possible ELF electromagnetic field effects on forest ecosystems requires the careful matching of plots to reduce variability among sites. Therefore, environmental factors that influence vegetation have been considered in selecting study sites. The Trees and Herbaceous Plants study design required plots to be located along a portion of the antenna, at the antenna ground, and at a control site located some distance from the antenna. Soil characteristics, microclimate, site history, and the vegetative community were carefully evaluated to insure as much similarity between test and control plots as possible. Field measurements for determining similarity among sites are shown in Table 1.

Selection of Ground and Antenna Sites.

The antenna site was located and established in 1983. Following an update on the location of the antenna ground in October 1983, a study site along the antenna ground route (T45N R29W Sec. 28) was selected for study. This 1.8 ha (4.5 ac) area was selected to be cleared of existing vegetation and planted with red pine. No overstory tree plots would be established since buffer strips would result in trees being too great a distance from the ground for meaningful studies (see 1983 Annual Report). Preliminary characterization work was started, but due to the late season only the soil description was completed. Evaluation of herbaceous vegetation completed selection criteria for this site in the spring of 1984.

Selection of Control Site.

In November 1983, we were informed by IITRI that our established control site (4C2) was unsuitable because background 60 Hz fields at this location differed by more than one order of magnitude from the 60 Hz fields

Table 1. Criteria Used for Selecting ELF Study Sites.

Trees

- *Species Composition
- *Basal Area
- *Diameter Distribution
- *Site Index

Ground Flora

- *Species Composition
- *Frequency
- *Crown Coverage

Soil Morphology

- *Horizon Identification
- *Horizon Thickness
- *Texture
- *Drainage
- *Presence or Absence of Earthworms
- *Rock Abundance

Site

- *Slope
- *Aspect
- *Landform
- *Habitat Type

at the antenna site (4T2). Much of the remaining fall, winter and ensuing spring was spent evaluating potential control sites. In May 1984 a control site was chosen following testing for field strengths by IITRI (Results of IITRI tests are in Appendix B). This site is located in Iron County south of Crystal Falls (T41N R32W Sec. 3). Physical descriptions of each site show minor variation in slope, aspect and elevation among sites while all sites are classified as being in the Acer-Quercus-Vaccinuim habitat type (Coffman, et al. (1983)) (Table 2).

Table 2. Description of ELF Study Sites.

Location	Ground T45N R29W Sec. 28	Antenna T45N R29W Sec. 28	Control T41N R32W SW 1/4 Sec. 3
Percent Slope	0-30%	7-15%	0-15%
Aspect Range	NW	W-NW	NW
Slope Position	Level to Crest of ridge	Crest of slope to mid-slope	Crest of slope to mid-slope
Elevation	445 M	454 M	420 M
Habitat Type	Acer-Quercus-Vaccinium	Acer-Quercus-Vaccinium	Acer-Quercus-Vaccinium

Tree Inventory

All trees with diameters greater than 10 cm were inventoried at the antenna and new control sites. Tree species, total height, DBH, and insect and disease damage was recorded for each tree. Diameters were measured to the nearest 0.1 cm using diameter tapes and heights were measured to the nearest 25 cm with a Sunto clinometer. From these measurements basal area and total tree biomass were calculated; the regression equations used in estimating total tree biomass for each tree species can be found in Appendix C. A summary of inventory data is shown in Table 3. A Kolmogorov-Smirnov two sample test was used to test the hypothesis of similar diameter distributions for each of the five species on the antenna and control sites. Diameter distributions for all species combined are shown in Figure 1.

Differences in diameter distribution were non-significant ($p > 0.10$) for bigtooth aspen (Figure 2), northern red oak (Figure 3), and paper birch (Figure 4). However, the diameter distribution for red maple was found to be significantly different ($p < 0.005$) with greater numbers of larger diameter trees at the antenna site (Figure 5). All distributions appeared to be unimodal. Lack of significant differences in diameter distribution is probably the result of small sample sizes, but average tree dbh are close (as seen in Table 3) with the exception of red maple.

While all species of interest are present at each site, they differ in numbers. For example, the number of red oak is much larger at the control and the number of red maple is larger at the antenna site (Table 3). However, there are sufficient numbers of similar sized trees of each species at each site to adequately evaluate growth between sites (See Element 3).

Figure 1. Number of stems by diameter class for the control and antenna sites.

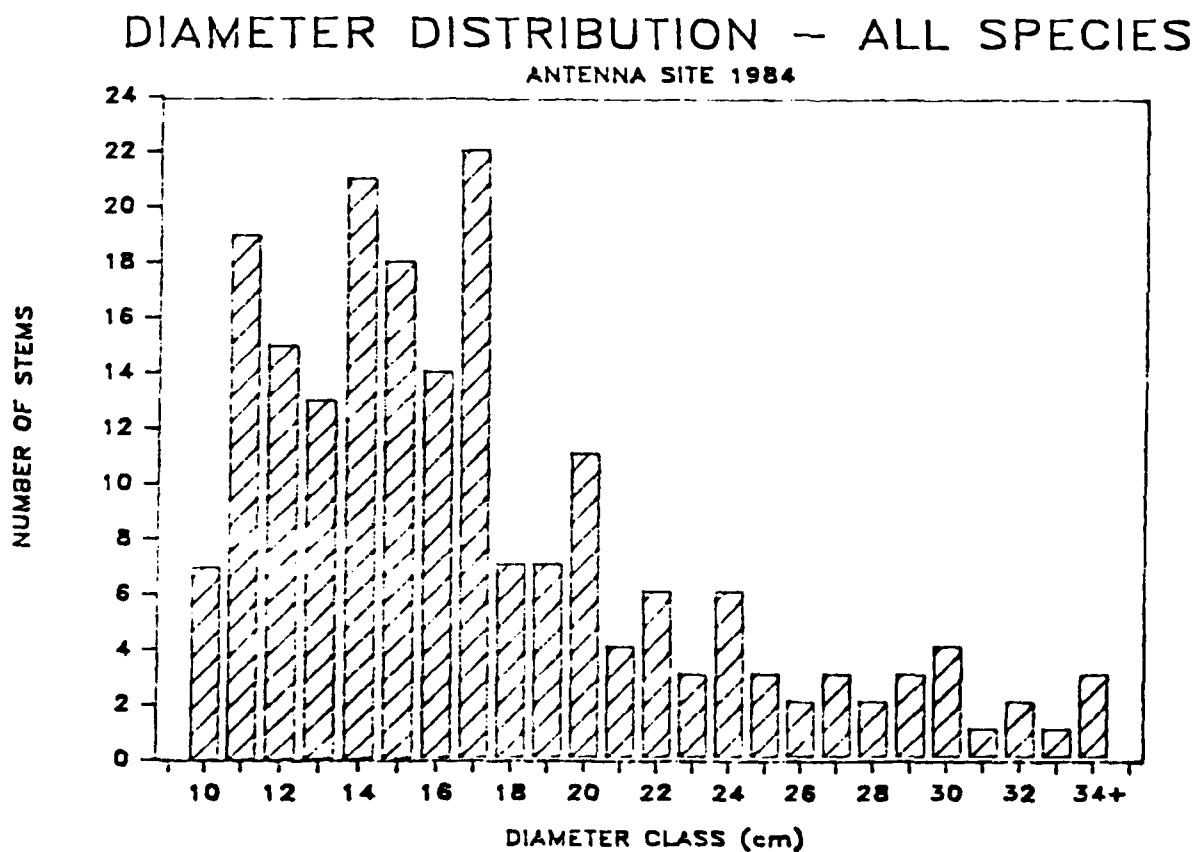
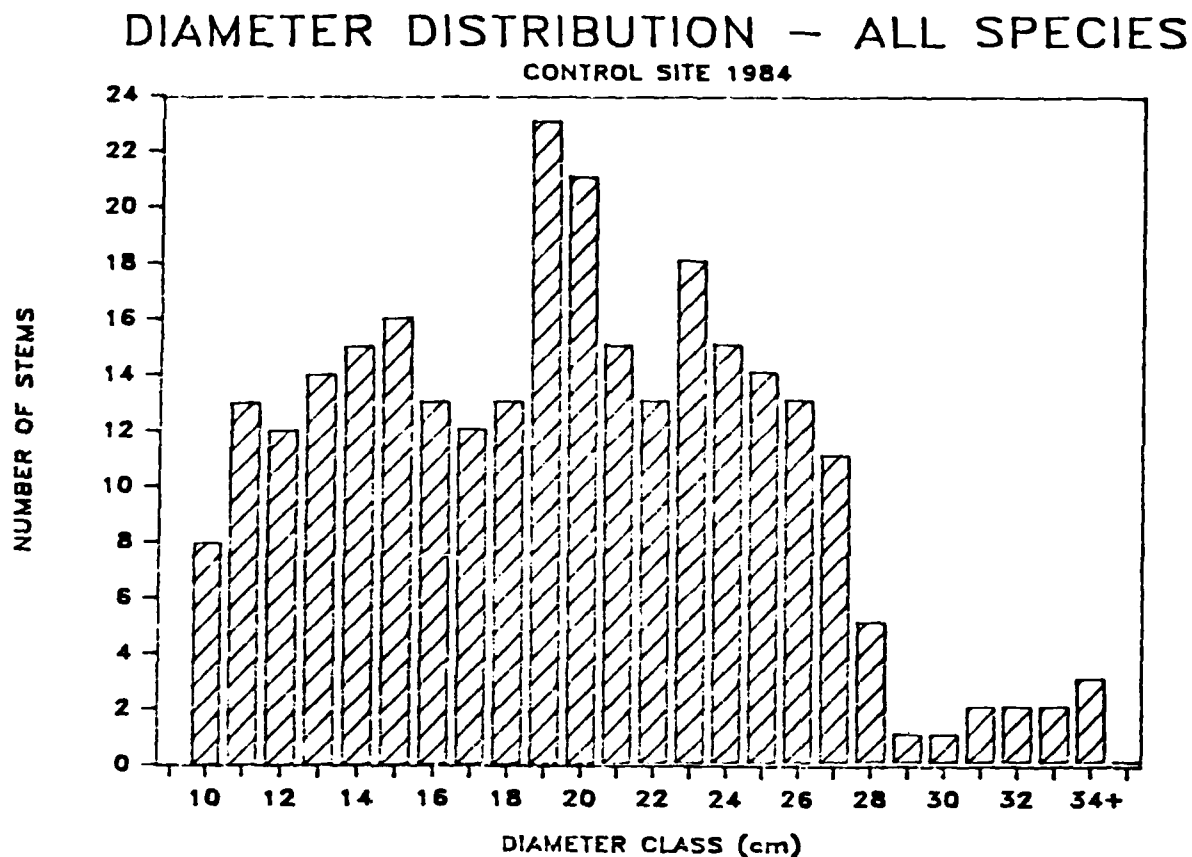
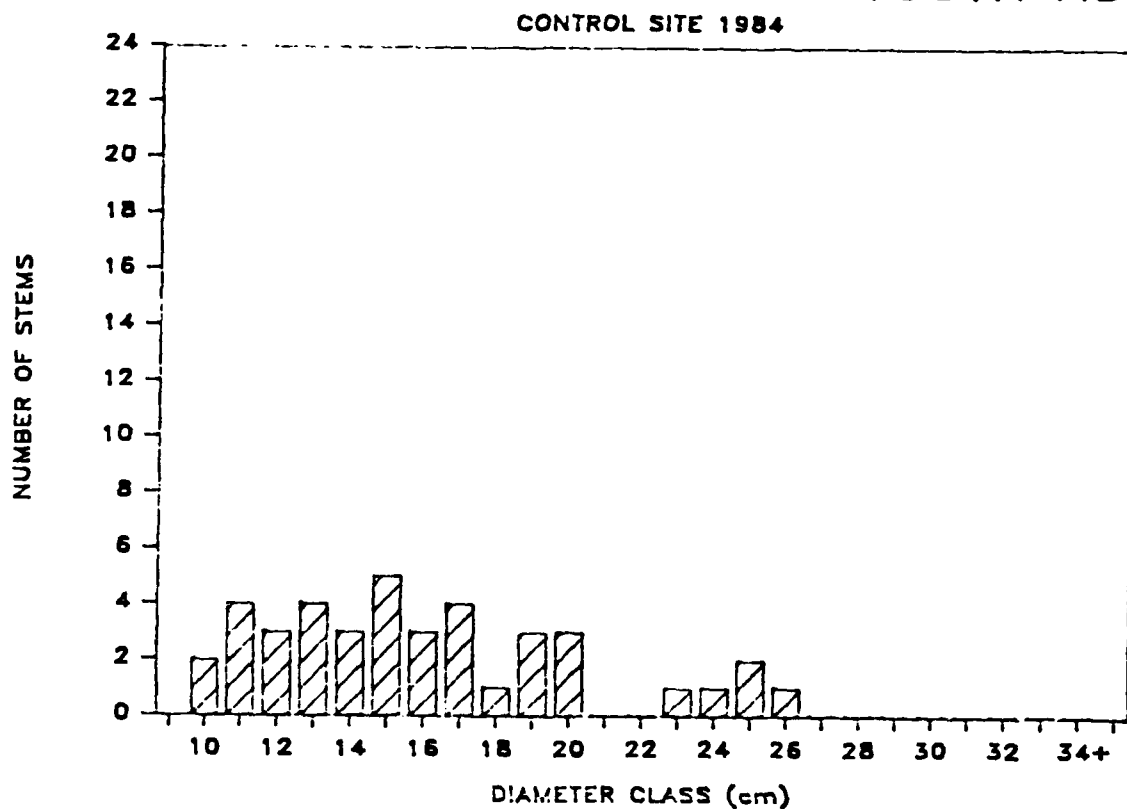


Figure 2. Number of Big Tooth Aspen stems by diameter class for control and antenna sites.

DIAMETER DISTRIBUTION—BIG TOOTH ASPEN



DIAMETER DISTRIBUTION—BIG TOOTH ASPEN

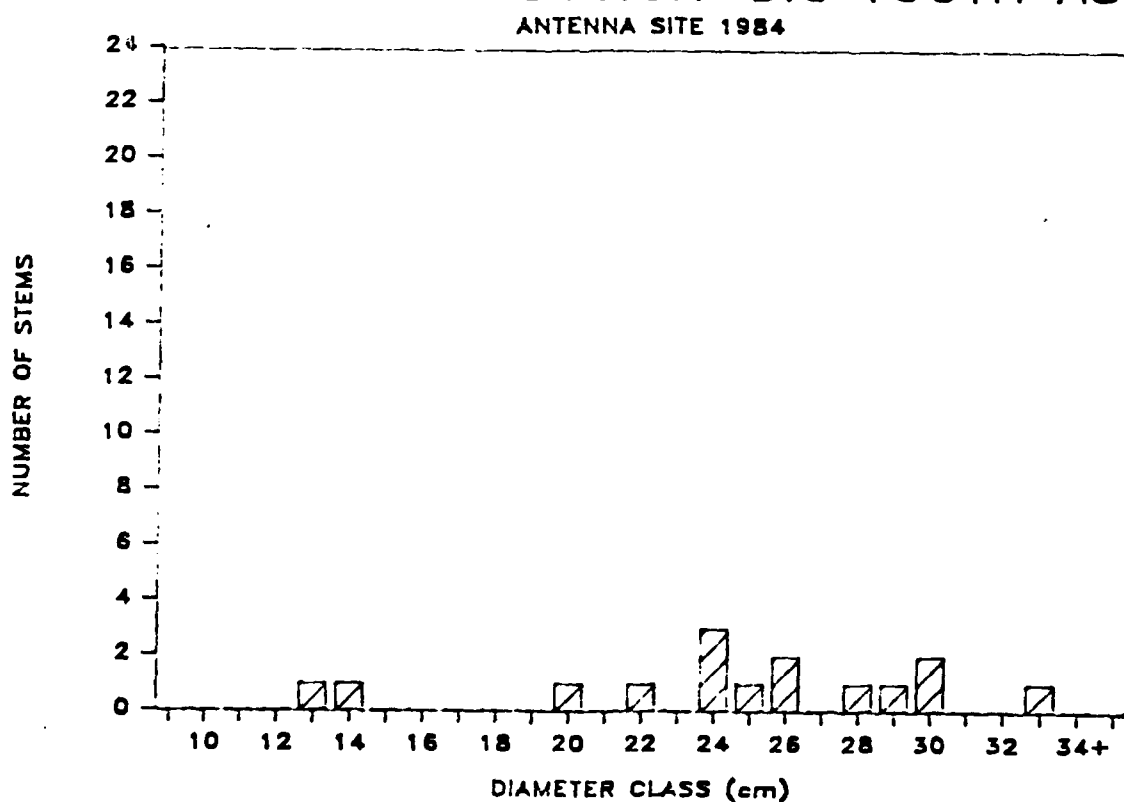


Figure 3. Number of Red Oak stems by diameter class for control and antenna sites.

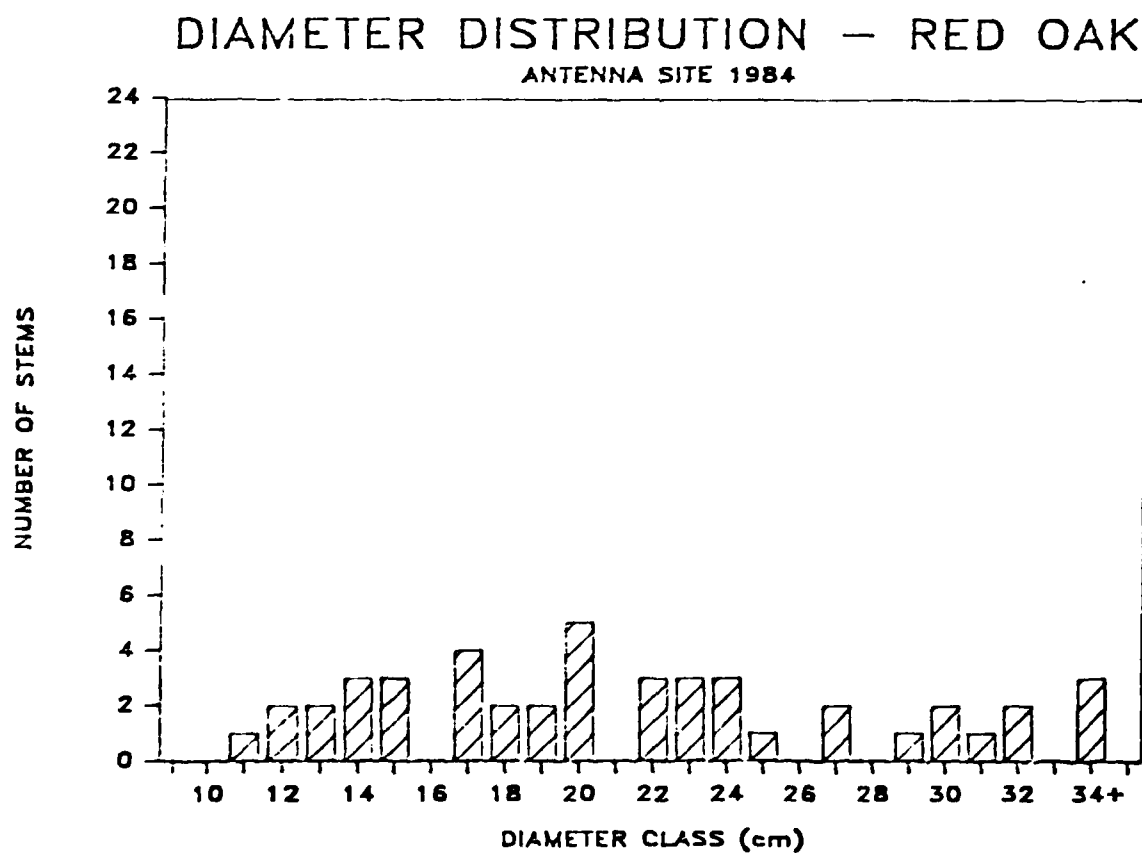
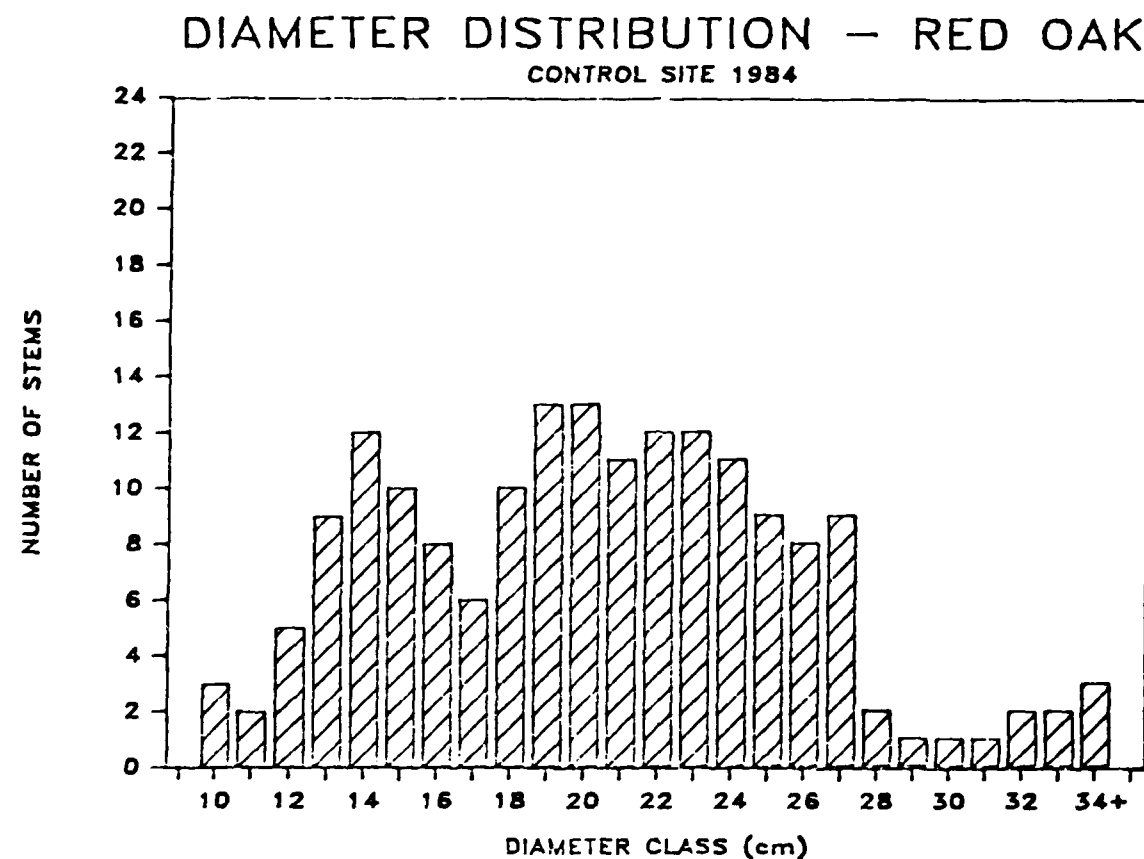


Figure 4. Number of Paper Birch stems by diameter class for the control and antenna sites.

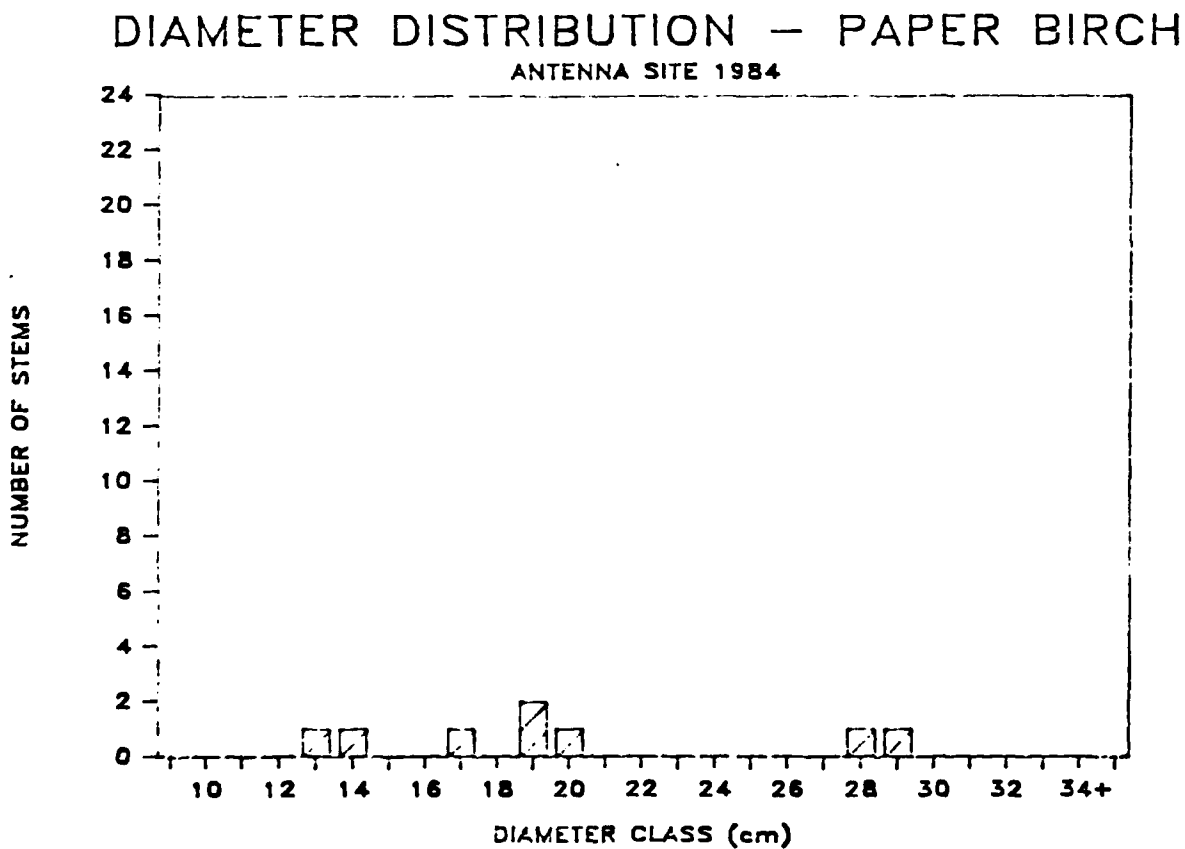
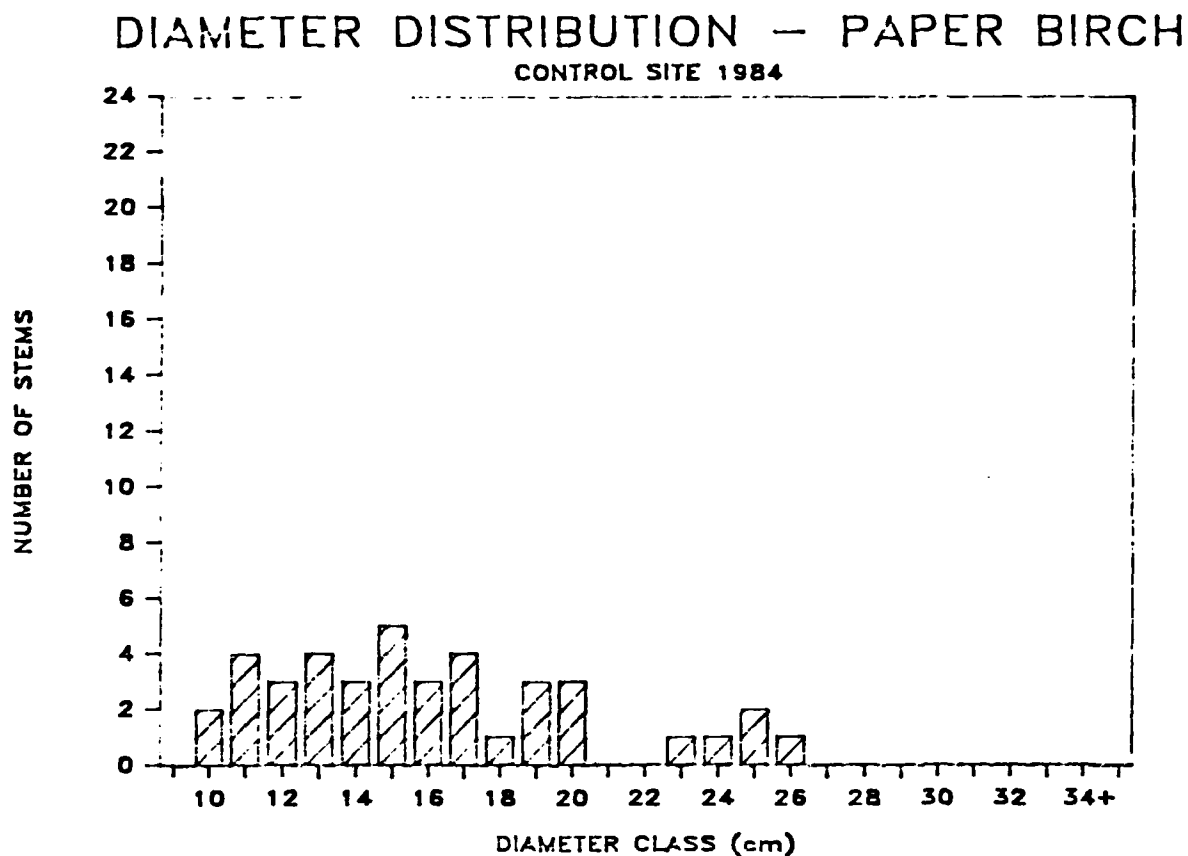
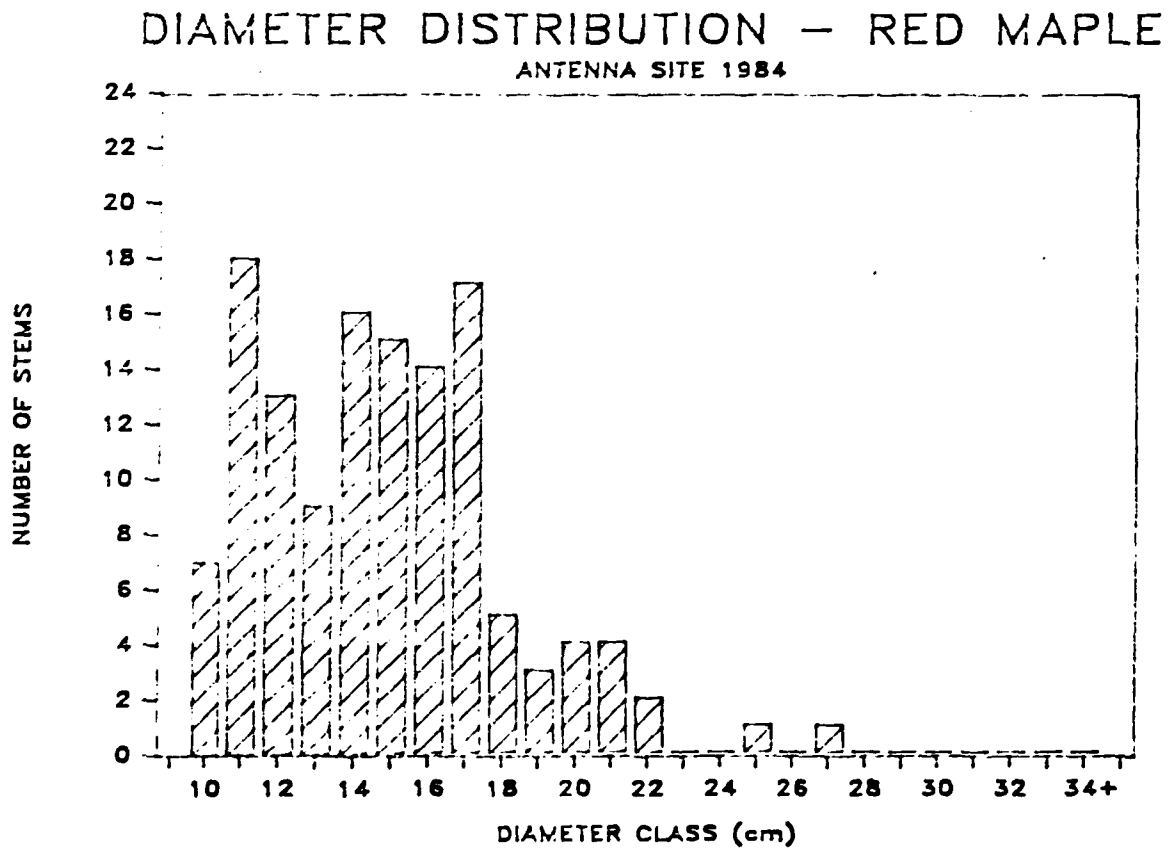
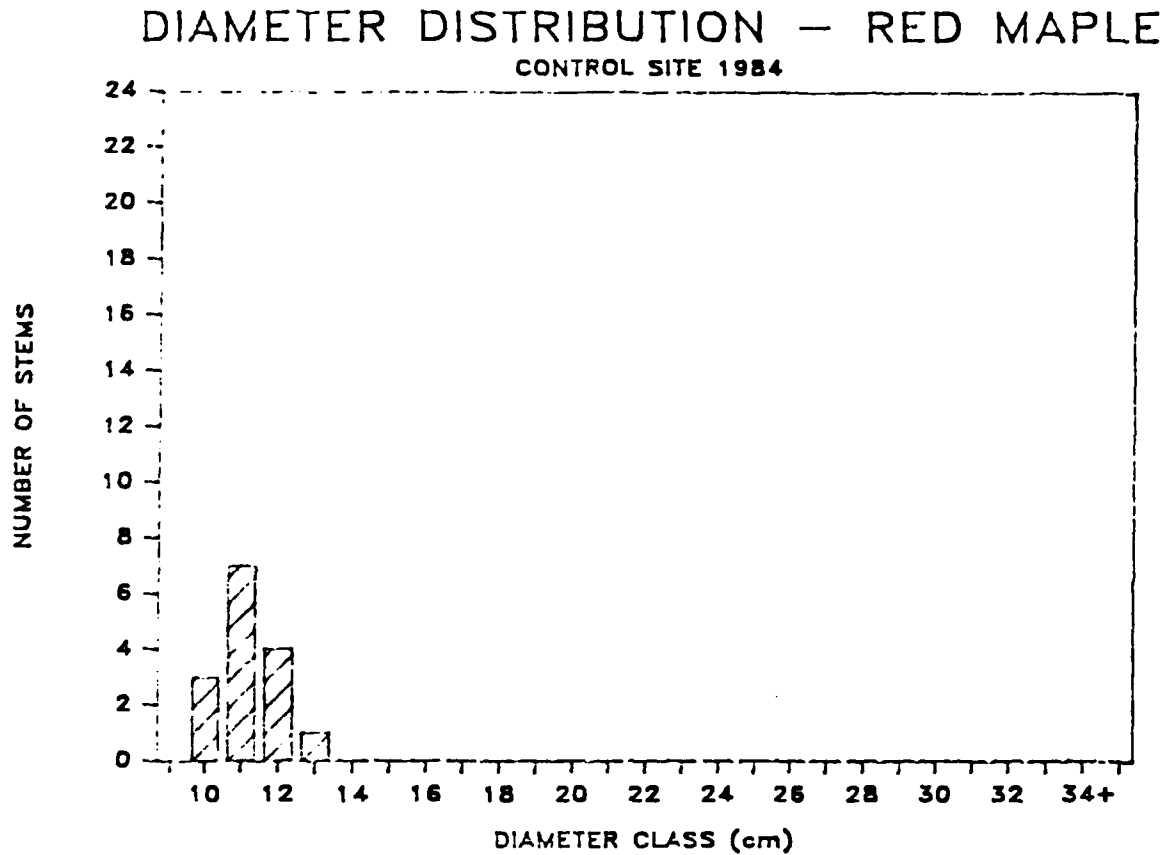


Figure 5. Number of Red Maple stems by diameter class for control and antenna sites.



Several similarity indices were applied to the tree inventory data to estimate similarities between the antenna and control sites (Mueller-Dombois and Ellenberg, 1974). The presence/absence of tree species was quantified using the Jaccard and Sorenson similarity indices where:

Jaccard

$$IS = \frac{\text{common species}}{\text{all species}} \times 100$$

Sorenson

$$IS = \frac{C}{1/2(A+B)}$$

Where C = #species common to each
A = #species on site A
B = #species on site B

The similarity of the two sites is not only a function of the common and unique species, but also of the amount of each species present. Similarity between the sites based on total tree biomass of the five species was quantified by the Ellenberg similarity index where:

Ellenberg

$$IS = \frac{C/2}{A + B + C/2} = 98\%$$

Where C = total biomass of common species
A = biomass unique to site A
B = biomass unique to site B

Taking into consideration quantitative differences in the abundance of each of the five species an index by Bray and Curtis (1957) using polar ordination was also considered. Consideration was made to number of stems and total biomass of each species occurring on each site. The two sites are 39.9% similar when looking at the abundance of stems per species and 65.8% similar when considering abundance of total biomass per species. Results from these tests show strong similarity between the control and antenna site

with indices of 80%, 89% and 98% for the Jaccard, Sorenson and Ellenberg tests respectively. Not as strong a similarity exists between the two sites with the Bray and Curtis index but this information will aid in explaining results in later analyses. The ground was not included in the test since there will be no studies of pole sized stands there. Sorenson's index differs from Jaccards in that it gives greater weight to the species that recur in the two test areas than to those that are unique to either area. Concerns in this study are based on similarities between sites and not uniqueness, thus Sorenson's index was given greater emphasis in site selection.

Soil Characterization

The soils on the new control site were sampled during the year. A complete soil profile description and bulk samples were obtained to characterize the morphological and compositional properties of the soil. Composite samples were obtained from each subplot to characterize the chemical variability within the rooting zone.

Reconnaissance investigations were conducted by making auger borings to 1.5 meters and noting a profile description. This was done to assess the uniformity of soil conditions across the study area. A soil pit was excavated at a representative location adjacent to the study plots and a detailed profile description made according to National Cooperative Soil Survey Standards. Bulk density samples and soil samples for physical and chemical analysis were obtained for each soil horizon. Composite samples were obtained from each plot for the upper 4 mineral soil horizons, by extracting five individual cores from a 2m² area. Two sets of composites were sampled from each plot. Each of these samples were returned to the Soil Research Laboratory at Michigan Technological University for physical and chemical analysis. Results are summarized in Appendix D.

Standard analytical techniques (Soil Conservation Service, 1972) were utilized for the chemical and physical analyses. Complete analyses were performed on the pit soil samples. The specific analyses for each pit soil sample are presented in Table 4.

The soil on the control site is classified as an Alfic Haplorthod, coarse-loamy, mixed, frigid. The soil is characterized by a coarse-loamy, spodic solum which overlies a stratified argillic horizon. The parent material on this site is a sandy glacial till. The spodic sequum has a moderate water holding capacity with low amounts of available nutrients, which is characteristic of spodosols. The argillic sequum, from 55 to 125 cm, has a good water holding capacity and fertility levels, with the base

Table 4. Chemical and Physical Analyses of Soil from ELF Study Sites.

Physical Analyses

Particle Size Distribution
Moisture Retention
Bulk Density

Chemical Analyses

Ca, Mg, Na, K, Fe, Al
pH, CEC, total N, C,
H

saturation varying from 70 to 80 percent.

The soils on the three study sites are classified differently (Table 5). These taxonomic differences reflect variations in both physical and chemical composition. Morphologically the control site exhibits the most development with the presence of the spodic and argillic horizons. The antenna site classifies in the same soil order, but it lacks the presence of the argillic horizon. The spodic development in these two soils are very similar, as exhibited by the horizon colors and indexes of accumulation. The ground site has a similar morphology when compared to the control site, exhibiting a weakly developed spodic horizon and thin illuvial horizon. However, the spodic sequum does not qualify taxonomically and the illuvial horizon does not meet the requirements of an argillic horizon. These variations result in its being classified into the Inceptisol soil order.

Compositional analysis of these soils demonstrate that there are minor variations in the chemical and physical properties. Table 6 presents the water holding capacity of the upper 100 cm of the soil profile for each study area. There are no differences between the ground and control plot. The difference between the antenna and control plot is attributed to the sandy solum and relatively lower carbon levels in the upper portion of the profile. Table 7 presents selected chemical properties from the upper profile for each of the study sites. The variations observed in these data indicate only small deviations from the control. The differences between the A horizons are a result of varying thicknesses and composition of the litter. The minor differences observed between these soils is not expected to affect the inherent site productivity of the study area. These sites would be expected to respond similarly to any environmental changes.

Table 5. Soil Classification of the Three ELF Study Sites.

<u>Site</u>	<u>Classification</u>
Ground	Typic Dystrocrept, sandy, mixed, frigid
Antenna	Entic Haplorthod, sandy, mixed, frigid
Control	Alfic Haplorthod, coarse-loamy, mixed, frigid

Table 6. Water Holding Capacity in the Upper 100 cm of Soil.

<u>Site</u>	Total Water Retention
	<u>cm</u>
Ground	3.96
Antenna	2.84
Control	4.00

Table 7. Comparison of Select Chemical Properties among ELF Study Sites.

Horizon	Antenna	Ground	Control
<u>Calcium</u> (meg/100 gms)			
A	6.9	9.8	3.4
E	0.2	1.0	Tr
Bs1	0.2	0.4	1.5
Bs2	0.1	0.3	1.1
<u>Nitrogen</u> (%)			
A	0.570	0.044	0.240
E	0.029	0.005	0.035
Bs1	0.028	0.005	0.034
Bs2	0.009	0.004	0.017
<u>Acidity</u> (meg/100 gm)			
A	40.6	19.3	19.1
E	1.8	4.5	3.4
Bs1	10.5	5.0	6.9
Bs2	3.8	1.2	3.4
<u>Cation Exchange Capacity</u> (meg/100 gms)			
A	50.7	18.8	23.4
E	1.9	3.4	3.9
Bs1	5.1	2.3	5.0
Bs2	1.7	1.3	2.9

Analysis of the composite samples from the control site is presented in Appendix E. Cross tabulations of selected nutrients are presented in Table 8. These data support the need for replicated, composite analyses for nutrients. The coefficients of variation of the presented data range from 10 to 100 percent. Depending on the accuracy required of these variables when they are utilized as covariates in the growth response analyses, additional sampling may be required. However, the observed variation in the data is typical for glaciated soils within the region. Comparison of the composite samples from the antenna site and control site demonstrated that there are significant differences in selected properties between the two study areas for the surface horizon (A), while no differences were observed between the subsurface horizon (Bsl) (Table 9). The analyses of the Bsl horizon corroborates the morphological observations. Similarly, the differences observed in the surface (A) horizon correspond with the variability of the forest floor on these sites.

The existing soil analyses may be sufficient to represent the conditions of the forested stands. However, variables to be used as covariates must be selected before this determination can be finalized. Inclusion of the soil properties for analyses in the reproduction studies should utilize data obtained from replicate-composite sampling to minimize the variance. Seedlings rely on the fertility of the surface horizons for their growth; given the observed variations in properties among sites, continued sampling is warranted for these studies.

Soil Characterization - Continued Research

Morphologically the soils on the three study sites are similar - the composition of the subsoil has also been demonstrated to be similar between study areas. However, significant variations between the surface horizon exist, as do specific variables within a study area. Accordingly, the following recommendations are made for continued research.

Table 8. Cross Tabulation of Selected Nutrients for Composite Samples from the A horizon on the Control Site (2 Replicates/Plot).

Plot Number	C ←	N →	Ca ←	Mg	Na	K	AL	CEC →
311 R1	5.42	.219	5.9	1.4	TR	0.7	0.4	19.8
311 R2	6.24	.298	6.0	1.7	TR	1.1	0.5	4.7
312 R1	6.53	.199	9.0	2.2	TR	0.6	0.1	23.5
312 R2	10.40	0.50	16.0			0.9	0.1	34.4
313 R1	6.30	.346	9.2	1.9	TR	0.5	0.2	5.8
313 R2	3.72	.151	8.5	1.7	TR	0.4	0.2	19.2
321 R1	6.23	0.277	9.9	2.2	TR	0.3	0.2	20.0
321 R2	7.79	0.189	5.3	1.4	TR	0.2	1.5	27.1
322 R1	7.14	0.302	8.0	1.9	TR	0.4	1.4	26.7
322 R2	6.54	0.316	9.3	1.8	TR	0.4	0.6	22.6
323 R1	6.05	0.297	5.5	1.2	TR	0.4	1.6	25.9
323 R2	9.29	0.437	10.1	2.0	TR	0.7	1.1	33.3
Mean	6.80	0.294	8.5	1.9	—	0.5	0.7	21.9
Std. Dev.	1.74	0.101	2.9	0.5	—	0.3	0.6	9.2
Coef. Var (%)	25.6	34.4	34.1	26.3	—	60.0	85.7	42.0

Table 8 con't. Cross Tabulation of Selected Nutrients for Composite Samples from the E horizons on the Control Site (2 Replicates/Plot).

Plot Number	C ← →		N ← →	Ca	Mg	Na	K	Al	CEC →	
311 R1	1.00		0.052	1.1	0.7	.1	0.2	1.0	6.2	
311 R2	1.03		0.059	1.6	0.5	TR	0.2	0.5	5.7	
312 R1	0.84		0.049	2.1	0.6	TR	0.2	5.8	7.3	
312 R2	0.85		0.083	2.2	1.0	.1	0.2	6.3	6.0	
313 R1	1.48		0.076	2.2	0.5	—	0.1	1.5	8.0	
313 R2	1.16		0.069	2.4	0.6	—	0.1	1.6	6.8	
321 R1	0.97		0.050	0.9	0.4	—	0.1	1.3	5.7	
321 R2	1.17		0.056	0.5	0.3	—	0.1	1.8	6.8	
322 R1	1.23		0.059	0.5	0.3	—	0.1	2.4	7.1	
322 R2	0.75		0.045	0.7	0.2	—	0.1	1.4	5.5	
323 R1	0.82		0.049	0.3	0.3	—	0.1	1.1	5.2	
323 R2	1.22		0.062	0.7	0.4	—	0.1	1.2	6.4	
Mean	1.02		0.059	1.22	0.5	—	—	2.2	6.4	
Std. Dev.	0.21		0.012	0.7	0.2	—	—	1.9	0.8	
Coef. Var (%)	20.6		20.3	61.4	40.0	—	—	86.4	12.5	

Table 8 con't. Cross Tabulation of Selected Nutrients for Composite Samples from the Bsl horizon on the Control Site (2 Replicates/Plot).

Plot Number	C		N		Ca		Mg		Na		K		AL		CEC	
	<	>	<	>	<	>	<	>	<	>	<	>	<	>	<	>
311 R1	0.62	0.037	1.2	0.3	TR	0.1	0.3	5.4								
311 R2	0.65	0.041	1.9	1.0	0.1	0.1	0.3	5.5								
312 R1	0.51	0.168	1.5	0.3	—	0.1	0.4	4.5								
312 R2	0.61	0.314	1.6	0.4	—	0.1	0.3	5.0								
313 R1	0.84	0.055	1.9	0.4	TR	0.1	1.4	6.0								
313 R2	0.89	0.127	1.9	0.4	—	0.1	1.2	6.4								
321 R1	0.58	0.036	1.1	0.4	—	0.1	0.8	5.4								
321 R2	0.73	0.066	1.2	0.5	—	0.1	0.7	5.9								
322 R1	0.68	0.039	0.8	0.3	—	0.1	0.4	5.4								
322 R2	0.80	0.050	0.9	0.3	—	0.1	0.6	6.4								
323 R1	0.54	0.031	0.7	0.2	—	0.1	0.9	5.1								
323 R2	0.62	0.042	0.9	0.3	—	0.1	1.9	5.8								
Mean	0.67	0.084	1.6	0.4	—	—	0.8	5.6								
Std Dev.	0.12	0.084	0.9	0.2	—	—	0.5	0.6								
Coef. Var (%)	17.9	100.0	56.3	50.0	—	—	62.5	10.7								

Table 9. Analysis of Variance for Selected Properties of the A and Bsl Horizons from the Control and Antenna Sites.
(F .05; 1, 13 df = 4.67).

Variable	Antenna	Control	F-Ratio	Significance
<u>A Horizon</u>				
Carbon (%)	11.36	7.17	11.32	S
Nitrogen (%)	0.50	0.30	9.58	S
Calcium (meg/100gm)	13.36	8.02	2.82	NS
CEC (meg/100gm)	37.5	25.9	4.7	S
<u>Bsl Horizon</u>				
Carbon (%)	0.72	0.66	0.56	NS
Nitrogen (%)	0.052	0.044	0.51	NS
Calcium (meg/100gm)	0.70	0.93	2.25	NS
CEC (meg/100gm)	6.19	0.46	0.32	NS

- (A) Determine those properties which will be used in the growth response analyses of the forested plots. Composite soil samples should then be collected bi-annually for analyses.
- (B) There is considerable variation in the properties of the surface soil horizon. To adequately address this variation with respect to the growth response of red pine seedlings, periodic composite sampling should be performed. Changes in macro-nutrients concentrations should be monitored through continued sampling of the A, E, Bs1 and Bs2 horizons. The sampling and analyses procedure represent a cost effective method to assess changes in nutrient concentrations that occur between plots.

To complete this initial phase of the soil characterization portion of the Plot Selection Element a summary report is being prepared which will contain all the pertinent soil data for the three study areas. Continued monitoring of soil nutrients will be transferred to the Tree Productivity element of this project.

Element 2. Ambient Monitoring

The terrestrial ambient monitoring program is designed to provide for the collection and analysis of climatological and soils data which affect plant growth processes. These parameters include precipitation, air temperature, relative humidity, solar radiation, soil temperature and soil moisture. The ambient monitoring element is being conducted to support the research elements within the tree, herbaceous and litter decomposition tasks. Accordingly, the ambient monitoring program has maintained a structure and design which is consistent with the data requirements of our entire research program. The appropriate elements should be referred to for discussions regarding the utilization of the ambient monitoring data.

System Configuration

Each of the study areas has been equipped with a Handar 540A data collection platform and a specified complement of sensors for each of the subplots. The main plot with the data collection platform contains the precipitation, air temperature, relative humidity, solar radiation, soil temperature and soil moisture; the subplots contain air temperature, soil temperature and soil moisture sensors. Appendix F presents the complete configuration for each platform.

The MET Board (Meteorological Data Processing Board) had to be modified on each platform to accomodate additional inputs for the soil moisture and soil temperature sensors. The modifications were done at the University after receiving a prototype from Handar, Inc. Modifications of one board on the ground and control platforms remains to be completed in April, 1985. The modification will accomodate an additional 3 channels each of soil moisture/temperature data on the ground platform and 3 air temperature channels and 2 soil temperature channels on the control platform. Data integrity should not be jeopardized since the system

configuration is redundant for the soil moisture/temperature and air temperature sensors.

System Operation

The ambient monitoring systems became operational in August 1984; complete operational status was achieved in November, 1984, with the installation of the snow pillows. Daily communications are accommodated through the National Environmental Satellite, Data, and Information Service (NESDIS), utilizing the GOES East Satellite. Each system transmits eight times daily, at three hour intervals. Sensor data is queried every 30 minutes, during the 3 hour period, and computed into a mean value by the platform micro-processor. Precipitation data is logged once, each three hour period. Accordingly, the transmitted data for each platform consists of a one dimensional array comprised of a three hour mean for each sensor channel, except precipitation which is logged. (A listing of each sensor channel and sensor specifications are contained in Appendix F).

The data retrieval procedure for the NESS down-link site is summarized in Appendix G. This procedure is now principally contained on an IBM-PC micro-computer. Our previous intentions (1983 Annual Report) were to utilize a VAX750 computer. However, subsequent evaluation of the data retrieval procedure demonstrated that the PC would provide more reliable operations at greatly reduced cost, and provide the flexibility of operations at multiple sites. Furthermore, the PC's are readily available at the Research Center and Department which can facilitate data utilization and processing.

Data summarizations are currently being provided to the project groups (as indicated in Appendix G). Additionally, software is currently being developed to provide independent analysis of the ambient data, in order to assess climatological patterns and variations between study areas.

Continued Research

Continued operation of the ambient monitoring system will provide required data for assessing variations in climatological events and soil properties between study areas. In addition to the routine operation of the system, the following aspects will be pursued:

- (A) Analyses of climatological events and resulting effects in the soil system. Specifically, relationships between soil moisture and temperature will be evaluated with respect to precipitation and air temperature. These analyses are unique to the Lake States Region and will provide the basis for research publications as well as complement the ELF Study.
- (B) Conduct studies for data validation of the soil moisture sensors and snow pillows. Evaluation of the soil moisture content gravimetrically. Additionally, the nylon resistance block sensor will be evaluated with respect to the current sensor. The snow pillow will be evaluated by periodically conducting a field survey with the 'Mont Rose Snow Sampler', to assess the snow pack depth and water equivalent. Consideration will also be given to the need for obtaining a 'snow triangle' will be considered for: 1. more simplified and reliable operations; 2. increased data resolution; 3. less susceptible to vandalism and rodent damage.

Element 3. Tree Productivity

Tree growth is sensitive to a variety of environmental disturbances. In order to detect any changes in growth due to site disturbance, accurate tree measurements are essential. The most widely accepted tree growth measurements are diameter at breast height outside bark (DBH) and height. Of these two growth variables, height is the more difficult to measure. The installation of permanent dendrometer bands on the stem of a tree allow measurement of minute changes (0.254mm) in diameter over a short time interval (Husch, et al., 1972). Two additional advantages in using DBH as a measurement of tree growth are the responsiveness of cambial activity to environmental effects (Smith, 1962) and the strong correlation existing between DBH and total biomass of the tree (Crow, 1978). Consequently, measurements of diameter increment will be the primary response variable for assessing ELF fields on stand growth. Tree height was used in initial stand characterization.

While DBH and height measurements can provide information on present stand production and a means to predict future productivity, the capacity of a stand to continue producing can be determined by monitoring tree reproduction and mortality. Stand structure, the distribution of trees by diameter classes, changes from year to year due to natural ingrowth (reproduction) and mortality of trees. Any environmental disturbances could produce an effect on these two factors; thus, natural changes need to be monitored and recorded in order to distinguish these from changes produced from site disturbances. Therefore, to achieve a complete picture of possible ELF effects on tree and stand production, DBH, height, ingrowth, and mortality will be measured.

In addition to tree productivity in pole-sized stand regeneration studies involving planted red pine seedlings were initiated this year at the antenna, ground and control plots. The necessity of such plots was fully described in last years report. The major justifications for planting are:

- 1) Field examination of the study sites show an inadequate number of conifers necessary for the ectomycorrhizal studies;
- 2) We are responding to Michigan DNR concerns on forest regeneration. Since young trees often exhibit rapid growth rates, possible ELF field effects on plants may be more easily detected;
- 3) Planting along the antenna ground will alleviate conflicts between the original study design and construction scheduling by allowing the establishment of plots for baseline studies before antenna ground installation commences;
- 4) The magnetic fields associated with antenna grounds rapidly decrease in strength over a short distance.

Consequently, any plot having a buffer strip of trees along the right-of-way would be too distant from the antenna ground to meet nominal field strength differences specified by IITRI in the original RFP.

In response to these constraints, a 4.4 acre area was clearcut along antenna ground #5 and red pine seedlings planted on a 1M X 1M spacing. This insured that once the ground is constructed, plots could be located as close as possible to the ground for maximum ELF field exposure. By establishing a seedling plantation in the cleared area, a buffer strip is not needed between the plots and the antenna right-of-way to eliminate edge effect is not needed. A 3.8 acre and 3.3 acre plantation were also established at the antenna and the control site.

Pole sized stands

Dendrometer band readings

Prior to the start of the growing season, permanent dendrometer bands were constructed and installed at DBH (4.5 feet or 1.4m above the ground) for all trees greater than or equal to 10 cm on both the Martel's Lake and the control sites. Methods for construction of the bands was given by Liming (1957). Bands were installed on 209 trees at antenna site and 275 trees at the control. Bands were read weekly beginning May 11 at the antenna site and June 10 at the control and continued until the start of fall leaf coloration. Due to the slow diameter growth in the fall, bands were read every other week until leaf fall was completed on October 17.

All data from these readings were entered directly into the Omnidata Polycorder in the field. The polycorder was interfaced with the IBM-PC microcomputer to transfer weekly data to a floppy disk for permanent storage. This procedure reduced error due to key punching, misreading or transferring to less than 1%.

All dendrometer band readings were taken in inches of circumference to the nearest 0.01 inch. Correction for initial slackness in these bands was made for the data based on the correction procedure developed by Auchmoody (1976) for black cherry (Prunus serotina). Several assumptions were made in using this method. His correction model was developed for trees whose bark was scraped smooth prior to dendrometer band installation. The trees banded in our study are young and have similarly smooth bark. Linear growth from the first reading to the point of slack retention was also assumed. The point of slack retention was subjectively picked for each tree and the full correction factor applied to every reading afterwards. If the tree did not show a measureable growth increment (less than 5 mm), then

the correction factor was not applied. This prevents overestimation of growth and possible "negative" growth in the following years.

In the coming spring (1985), five trees of each species will be banded a second time. Information on band slack will be collected and a correction factor developed specifically for the tree species in this study.

Growth Analysis

Analyses of tree diameter growth will be approached in two ways; Covariate analysis will be used to determine if there is any change due to ELF fields in average seasonal (May through October) or yearly diameter growth. At the same time, regression models will be developed and tested for changes due to ELF effects in diameter growth at some specific time period; in other words, it will test for changes in growth rates. Though overall average seasonal diameter growth may not change due to ELF fields, the rates of growth within the year and the relationships with stand and climatological variables may alter and could be detected. Both analyses will incorporate stand and climatological data. Tree growth does fluctuate from year to year depending on weather and stand conditions and ELF antenna effects may be subtle. By examining tree growth from these two different vantages, an ELF effect may be more discernable.

Covariate analysis will be used to test the hypothesis that there is no change in average seasonal (May through October) diameter growth for any of the five tree species under consideration. Assumptions necessary for valid use of analysis of covariances (Cochran, 1980) are:

- 1) the covariates are fixed, measured without error, and independent of treatments
- 2) after the removal of block and treatment differences, the response of the dependent variable from the covariates is linear and independent
- 3) the residuals are normally and independently distributed with mean zero and equal variance.

Before analyses can be completed all assumptions will be tested to determine if any violations have occurred. The relationship of the dependent variable with that of the covariates may or may not be linear, but a linear relationship is often a reasonably good approximation for a nonlinear relationship provided that the range in values of the independent variables or covariates is not too large (Cochran, 1980). Tests will be made to check for linearity of the response variable and to determine how robust this relationship is. Depending on the sensitivity of a violation in linearity or in unequal variances, transformation of variables can linearize variables as well as stabilize variances and could aid in meeting the assumptions of the analysis. If the data for this study is nonrobust, then alternative covariates will be used to adjust the analyses.

Using the 1984 spring and fall measurements one average seasonal diameter growth measurement is available on both the antenna and control sites. Although an average dbh measurement was calculated for the antenna site in the summer of 1983, many of the trees sampled were not the same ones banded in 1984. Consequently, growth for an individual tree could not be established to later determine the variance of growth in that year. The control site is new this year and no previous diameter information was available.

In order to calculate the sensitivity of our analysis based on one average growth measurement for each site, several assumptions were made. We assumed S_x^2 (variance of the growth in 1984) was equal to S_y^2 (variance of growth in 1985) on a given site for a given species. We also assumed that if the antenna is fully operational in 1988 we will have four years of baseline data. From this we can estimate the variance of growth from year to year and calculate the sensitivity of our analyses. The variance of growth from year to year can be estimated as

$$S = \text{Var} = S_x^2 + S_y^2 - 2 S_{xy}$$

where S_x^2 and S_y^2 are as previously defined and S_{xy} is the covariance of the two years of growth. Now

$$R = \frac{S_{xy}}{\sqrt{S_x^2 S_y^2}}$$

where R is the linear correlation or measure of the degree to which the two growth estimates vary linearly together; $R=1$ is high linear correlation and $R=0$ is no linear correlation. For varying values of R , Var can be estimated and the sensitivity of the analyses or the bounds within which our estimation is possible are then calculated as

$$n = \frac{(t_{.05, n-1}^2)(S^2)}{E^2}$$

or

$$E = \sqrt{\frac{t_{.05, n-1}^2 S^2}{n}}$$

where

$t_{.05, n-1} = 3.182$ = the value of the student's t-statistic at

$\alpha = 0.05$ with $(n-1=3)$ degrees of freedom

$S^2 =$ Var = the estimated variance of growth between years

$n =$ number of years of baseline data

$E =$ the bounds within which estimation of growth is possible

Table 10 illustrates the varying bounds of estimation for different correlations, R . The interval or bounds of estimation is much smaller when growth from year to year is highly correlated and one would expect a high correlation between the growth in one year to that of the next year. A second consideration is that no climatological data has been added as covariates; this additional information should reduce the variation of growth between years, so that even if the correlation was not as high, the covariates additional information should help reduce the variance. Finally, if more than four years of baseline data is obtained, then the t-value in the sensitivity test is reduced. By adding just one additional year reduces the percentage of the mean for red maple and northern red oak on the antenna site from 19.8 to 15.5 and 20.2 to 15.8 respectively. The table illustrates gross estimates of the bounds of estimation which can be determined from the available data.

Table 10 Sensitivity tests based on correlation coefficient, R, and estimated variance Var.

Species	Antenna Site				Control Site			
	R ^{1/}	Var ^{2/}	E ^{3/}	Percentage of Mean	R	Var	E	Percentage of Mean
Big Tooth	0.99	7.06X10 ⁻⁶	4.23X10 ⁻³	1.16	0.99	2.25X10 ⁻⁴	2.38X10 ⁻²	8.73
	0.90	6.99X10 ⁻⁵	1.33X10 ⁻²	3.64	0.90	2.25X10 ⁻³	7.54X10 ⁻²	27.67
	0.50	3.49X10 ⁻⁴	2.97X10 ⁻²	8.13	0.50	1.12X10 ⁻²	1.68X10 ⁻¹	61.65
	0.25	5.25X10 ⁻⁴	3.65X10 ⁻²	9.99	0.25	1.68X10 ⁻²	2.06X10 ⁻¹	75.59
Northern Red	0.00	6.99X10 ⁻⁴	4.21X10 ⁻²	11.53	0.00	2.24X10 ⁻²	2.38X10 ⁻¹	87.34
	0.99	3.89X10 ⁻⁴	3.13X10 ⁻²	20.18	0.99	1.36X10 ⁻⁴	1.85X10 ⁻²	13.99
	0.90	3.89X10 ⁻³	9.73X10 ⁻²	62.73	0.90	1.36X10 ⁻³	5.87X10 ⁻²	44.40
	0.50	1.94X10 ⁻²	2.21X10 ⁻¹	142.49	0.50	6.80X10 ⁻³	1.31X10 ⁻¹	99.09
Oak	0.25	2.91X10 ⁻²	2.71X10 ⁻¹	174.73	0.25	1.02X10 ⁻²	1.60X10 ⁻¹	123.59
	0.00	3.89X10 ⁻²	3.13X10 ⁻¹	201.001	0.00	1.36X10 ⁻²	1.85X10 ⁻¹	142.90
	0.99	1.20X10 ⁻⁴	174.10 ⁻²	17.05	0.99	8.85X10 ⁻⁵	1.49X10 ⁻²	17.93
	0.90	1.20X10 ⁻³	5.51X10 ⁻²	54.02	0.90	8.85X10 ⁻⁴	4.73X10 ⁻²	56.91
Paper Birch	0.50	6.03X10 ⁻³	1.23X10 ⁻¹	120.59	0.50	4.42X10 ⁻³	1.05X10 ⁻¹	126.35
	0.25	9.05X10 ⁻³	1.51X10 ⁻¹	148.04	0.25	6.63X10 ⁻³	1.29X10 ⁻¹	155.23
	0.00	1.20X10 ⁻²	1.74X10 ⁻¹	170.59	0.00	8.84X10 ⁻³	1.49X10 ⁻¹	179.30
	0.99				0.99	1.92X10 ⁻⁴	2.20X10 ⁻²	12.84
Quaking Aspen	0.90				0.90	1.92X10 ⁻³	6.97X10 ⁻²	40.68
	0.50				0.50	9.62X10 ⁻³	1.56X10 ⁻¹	91.06
	0.25				0.25	1.44X10 ⁻²	1.90X10 ⁻¹	110.92
	0.00				0.00	1.92X10 ⁻²	2.20X10 ⁻¹	128.43
Red Maple	0.99	1.69X10 ⁻⁴	2.06X10 ⁻²	19.85	0.99	1.23X10 ⁻⁴	1.76X10 ⁻²	9.59
	0.90	169.10 ⁻³	6.54X10 ⁻²	63.01	0.90	1.23X10 ⁻³	5.57X10 ⁻²	30.28
	0.50	8.46X10 ⁻³	1.46X10 ⁻¹	140.66	0.50	6.16X10 ⁻³	1.24X10 ⁻¹	67.43
	0.25	1.26X10 ⁻²	1.76X10 ⁻¹	169.56	0.25	1.08X10 ⁻²	1.65X10 ⁻¹	89.72
	0.00	1.69X10 ⁻²	2.06X10 ⁻¹	198.46	0.00	2.46X10 ⁻²	2.49X10 ⁻¹	135.69

1/ correlation coefficient

2/ variance of growth from year to year

3/ bounds of estimation

Seasonal growth (from May to October in 1984) for all species within each diameter class is illustrated in Figure 6. Histograms and data summary sheets for diameter growth of each species are contained in Appendix H. Tests were made for differences between the antenna and control site in average seasonal growth rates of each species. These tests did not include any covariates at this time, but compared average seasonal growth of a given species on each site for this year. Average seasonal growth was calculated from the June 7th or 9th reading until the end of the growing season in October to prevent any additional error resulting from growth not measured on the control site prior to this date. There was no significant difference in average seasonal growth of either northern red oak or paper birch ($p < .05$). There was a significant difference in average seasonal growth of both bigtooth aspen ($p > .05$) and red maple ($p > .05$). The results are found in Table 11.

Table 11. Results of comparing average seasonal growth by species between the antenna and control sites.

Species	\bar{x}_{ab} 1/	S_{ab}	n_{ab}	\bar{x}_c 2/	S_c	n_c	t- 3/ value
BTA	0.3652	0.0187	15	0.2725	0.1057	26	3.35*
NRO	0.1551	0.1395	45	0.1322	0.0825	175	1.41
PB	0.1020	0.0777	8	0.0831	0.0665	40	0.71
QA	—	—	—	0.1713	0.0981	19	—
RM	0.1038	0.0920	129	0.1839	0.0785	15	-3.23*

1/ Mean, standard deviation, and sample size of growth on the antenna site.

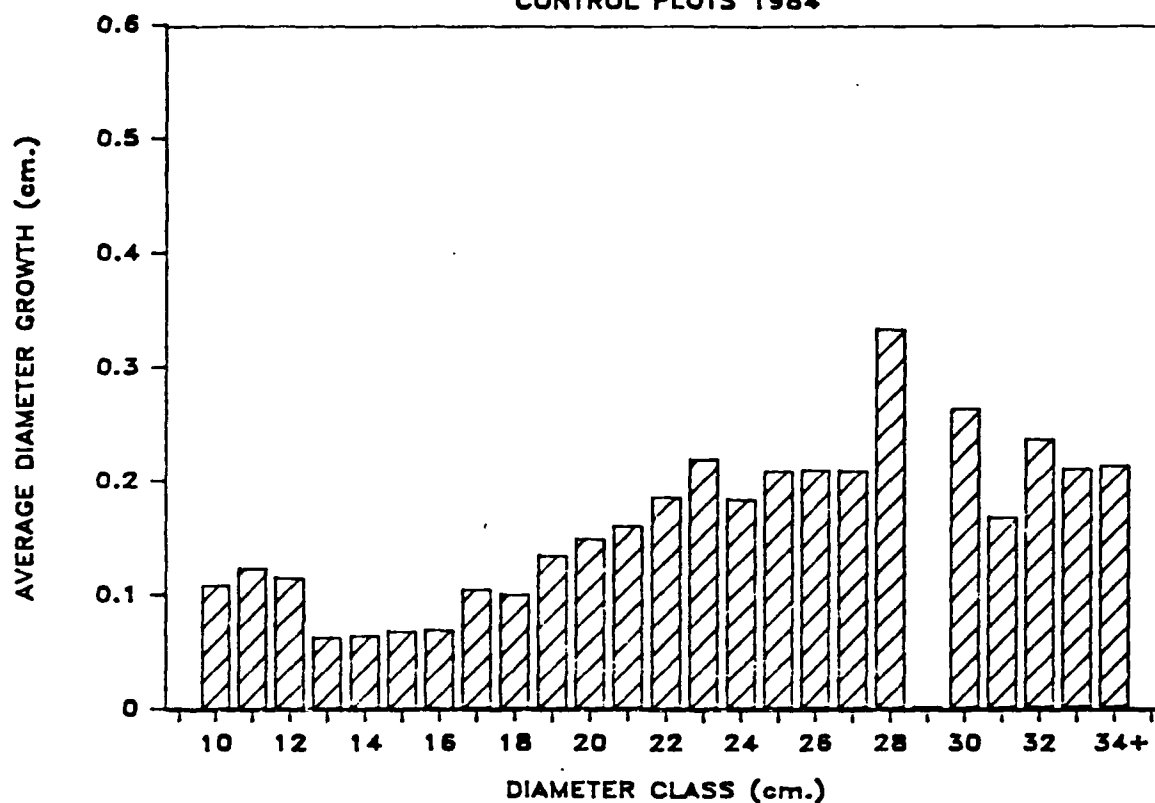
2/ Mean, standard deviation, and sample size of growth on the control site.

3/ t-value resulting from test of difference between two means at $\alpha = 0.05$ level with $t_{(n_1-1)+(n_2-1)}df$.

Figure 6. Seasonal diameter growth by diameter class for all species at the control and antenna sites.

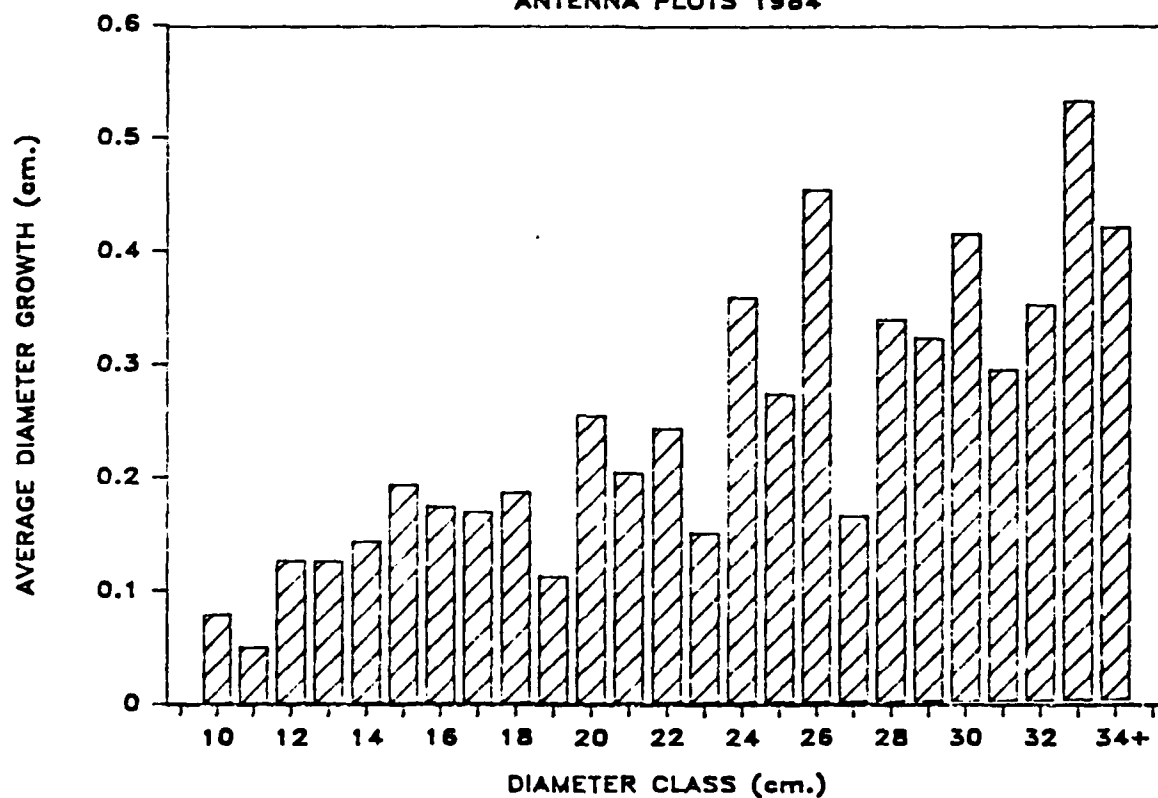
SEASONAL GROWTH — ALL SPECIES

CONTROL PLOTS 1984



SEASONAL GROWTH — ALL SPECIES

ANTENNA PLOTS 1984



Regression analysis will be used to study changes in growth rate over time a. Multiple regression models will incorporate stand and climatological variables as they affect diameter growth in the future. These models will be tested to determine if there is any change in the relationships of these variables on diameter growth due to ELF effects (Reynolds, 1984). Though overall average seasonal diameter growth may not change due to ELF effects, the rates of growth within the year and the relationship with site and climatological variables may alter and could be detected.

An initial multiple regression model for each species will be developed from this year's data and will be of the form

$$y_t = a_0 + a_1x_1 + a_2x_2 + \dots a_kx_k$$

where

y_t = diameter growth of a tree at time t

x_i = selected stand or ambient variable ($i=1,2,\dots,k$)

a_i = estimated coefficients ($i=0,1,\dots,k$)

Because there will almost certainly be interactions among the environmental factors being considered for the model, consideration will be given to multicollinearity. If multicollinearity is detected among the independent variables then the regression analysis will be modified accordingly.

If a nonlinear relationship is more appropriate then steps will be taken to fit a non-linear model. The model will be reevaluated and parameters readjusted each year thus allowing for a wider range in data;

with ambient variables included in the model with errors associated varying weather patterns can be accounted for. One model will be developed. Growth Information collected after the antenna has become operational will be used to evaluate the model to determine if the same trends hold true.

For both the covariate and the multiple regression analysis, the first step is to determine which of the stand and climatological variables are correlated and how they are correlated with average diameter growth of a given species. Covariates reflecting individual tree characteristics presently under consideration are initial tree DBH and tree crown ratio or a crown competition factor. Stand variables include basal area per acre, trees per acre and average stand diameter. Measures of stand density are being considered instead of stocking levels because stand density is a quantitative measurement of a stand while stocking levels are merely relative terms. Ambient variables now being considered are averages for the week before as well as the week during the tree's dendrometer band reading. They include average daily temperature, daily precipitation, daily soil temperature at 5 cm, 10 cm, and 100 cm depth, daily soil moisture at 5 cm, 10 cm, and 100 cm depth, and total precipitation. Data received from the ambient monitoring study did not begin until August 2, 1984. By this point approximately 80% of average diameter growth of each species had already taken place (Table 12). The relationships of these ambient variables are derived from the last 20% of average yearly diameter growth which has slowed down considerably by this point. This gave lower correlation values, as seen in Table 13. There are few strong linear correlations and graphs of these climatological variables yield little additional information. Models will be developed from this data, but should be more accurate in their

estimation of average diameter growth after the addition of next year's data. The correlations in Table 13 provide an initial view of the relationships of tree and climatological variables. Subsequent combinations and transformations of these and additional variables (e.g. Soil data, insolation, degree days etc.) will be examined to identify those most related to tree growth.

Table 12. Percentage of average diameter growth completed for each species prior to August 2, 1984.

<u>Site</u>	<u>Big tooth Aspen</u>	<u>Northern Red Oak</u>	<u>Paper Birch</u>	<u>Red Maple</u>	<u>Quaking Aspen</u>
Antenna	80.4	81.3	86.2	76.1	—
Control	83.9	85.0	90.9	82.4	84.7

Table 13. Correlation matrix of variables to be incorporated in both the covariance analysis and the multiple regression model.

	Species	WBH	Growth	Precip	Air Temp	Soil Temp	Soil Temp	Soil Moist	Total	Previous Growth	Previous Precip	Previous Air Temp	Previous Soil Temp	Previous Soil Moist	Previous Total
Species	1.0000														
WBH	-0.2957	1.0000													
Growth	-0.0404	.1083	1.0000												
Precipitation	0.0000	0.0000	-.0142	1.0000											
Air temp	0.0000	0.0000	.2227	-.0037	1.0000										
Soil temp 5 cm	0.0000	0.0000	.2174	.1206	.9780	1.0000									
Soil moist 5 cm	0.0000	0.0000	-.0936	-.2706	.1272	.2065	1.0000								
Total Precip	0.0000	0.0000	-.0316	.9879	-.1154	.0104	.2070	1.0000							
Previous growth	-0.0446	.1163	.1303	-.0132	.2500	.2616	.2058	-.0354	1.0000						
Previous	0.0000	0.0000	-.1074	.0179	.1199	.0469	.2738	-.0390	1.0000						
Previous Air Temp	0.0000	0.0000	.1131	.3951	.6509	.7188	.6598	.3039	-.0574	1.0000					
Previous Soil temp	0.0000	0.0000	.1140	.3838	.7325	.8409	.6339	.2779	.0562	.9824	1.0000				
Previous Soil Moist	0.0000	0.0000	-.1946	-.2112	-.4024	-.3708	-.2857	-.1867	.2104	-.0294	-.0024	1.0000			
Previous Total	0.0000	0.0000	-.1059	-.0341	.0955	.0098	.2238	-.0853	.9926	-.1447	-.0232	.1941	1.0000		
Precip															

* Previous values are those calculated for the time interval prior to the present time period.

Seedling Growth Studies

Land Clearing and planting

Areas to be cleared for establishment of red pine plantations were identified in the spring and cutting boundaries marked. Michigan DNR personnel cruised all sites for volume estimates and set specification for use of each site. Although IITRI measured background field strengths on May 15 and found them to be acceptable, legal concerns within the university caused substantial delays in plot establishment. Cruise volumes appear in Appendix I. Robert Minerick, Inc. of Sagola, a land clearing contractor, was chosen from several logging firms to clear the plantation plots. Work began on June 16 at the ground site. A feller-buncher was used to harvest trees and two grapple skidders used to transport whole trees to the landing. Sawyers delimbed trees before being skidded to a slasher where they were cut into eight foot lengths and decked. Any brush or slash that remained on the study sites was removed by chain saw or brush cutter by MTU personnel. Clearing was then started at the antenna and control sites and completed on June 27. Log decks were measured to determine the amount of material removed and results appear in Table 14.

Red pine planting was started at each site immediately following the clearing operation. Seedlings (3-Ø stock from a Dickinson County seed source) were obtained from the USDA Forest Service Touney Nursery in Watersmeet, Michigan. To keep seedlings healthy during the planting period, seedlings were refrigerated until planted. The seedlings were stored in a cooler at a milk ranch near Channing, Michigan and moved to the planting site daily in an insulated trailer packed with ice. A professional tree planter was contracted to expedite planting at the late date, to ensure the greatest uniformity in planting, and to maximize the chances for seedling survival. Michigan Tech personnel transported seedlings and supervised the

planting effort. Twenty-one thousand (21,000) red pine seedlings were hand planted on the sites at a 1 X 1 meter spacing. A summary of plot clearing and planting operations appears in Table 15.

Table 14. Volumes of wood material (cords) removed during plot clearing*.

Aspen Pulp	85.2	96.5	54.5
Hardwood Pulp	60.2	70.8	114.0
Oak Sawbolts	4.5	4.5	6.3
Pin Sawbolts	<u> </u>	11.2	2.2
Mixed Sawbolts	5.8	<u> </u>	<u> </u>
Total	155.7	183.0	177.0

* Includes 100' wide right-of-way at test sites, wood less than 4" top diameter inside bark and top wood of logs used for pulp.

Table 15. Summary of plot clearing operations
1984

	Clearing Started	Clearing Completed	Acres Cleared	Volume Removed (cdis)	Planting Started	Planting Completed	Number of Seedlings Planted
Ground	6/18	6/20	4.4	155.8	6/20	6/22	8,000
Antenna	6/19	6/22	3.8	183.0	6/22	6/25	7,000
Control	6/22	6/27	3.3	177.0	6/27	6/30	6,000
			<u>11.5</u>	<u>515.8</u>			<u>21,000</u>

Seedling Moisture Stress

Plant moisture stress (PMS) analyses began prior to planting to monitor the condition of seedlings during transport and planting. Following planting, it was used as an indicator of seedling adaption to the planting sites and an aid to determining the need for irrigation to insure acceptable survival. It will also be used as a covariate in seedling growth analyses to help quantify seedling vigor and growth since one of the most serious environmental stresses in the antenna area is seasonal drought.

Starting on July 12 and continuing every 2 weeks until the end of the growing season (only one sampling was done in October), 135 randomly selected seedlings (15 per plot) were severed near the root collar and tested for xylem water potential using a plant moisture stress meter (pressure bomb). Readings were taken in the pre-dawn hours of the morning to allow the maximum time possible for plant recovery from daily moisture stress. Prior to sampling, basal diameter and height (to top of candle) were measured, and seedling condition estimated. Seedling condition is a ocular evaluation regarding the percentage of brown needles on a seedling using the following scale: 1=0-25%, 2=25-50%, 3=50-75%, and 4=75-100%. The remaining stem and roots were excavated the afternoon following PMS sampling for laboratory analysis including mycorrhizae evaluation (Element 7) and determination of aboveground/belowground biomass (shoot-root ratios). Foliar samples from the initial planting stock and PMS samples taken in late October are being analyzed for concentrations of N, P, K, Ca, Mg.

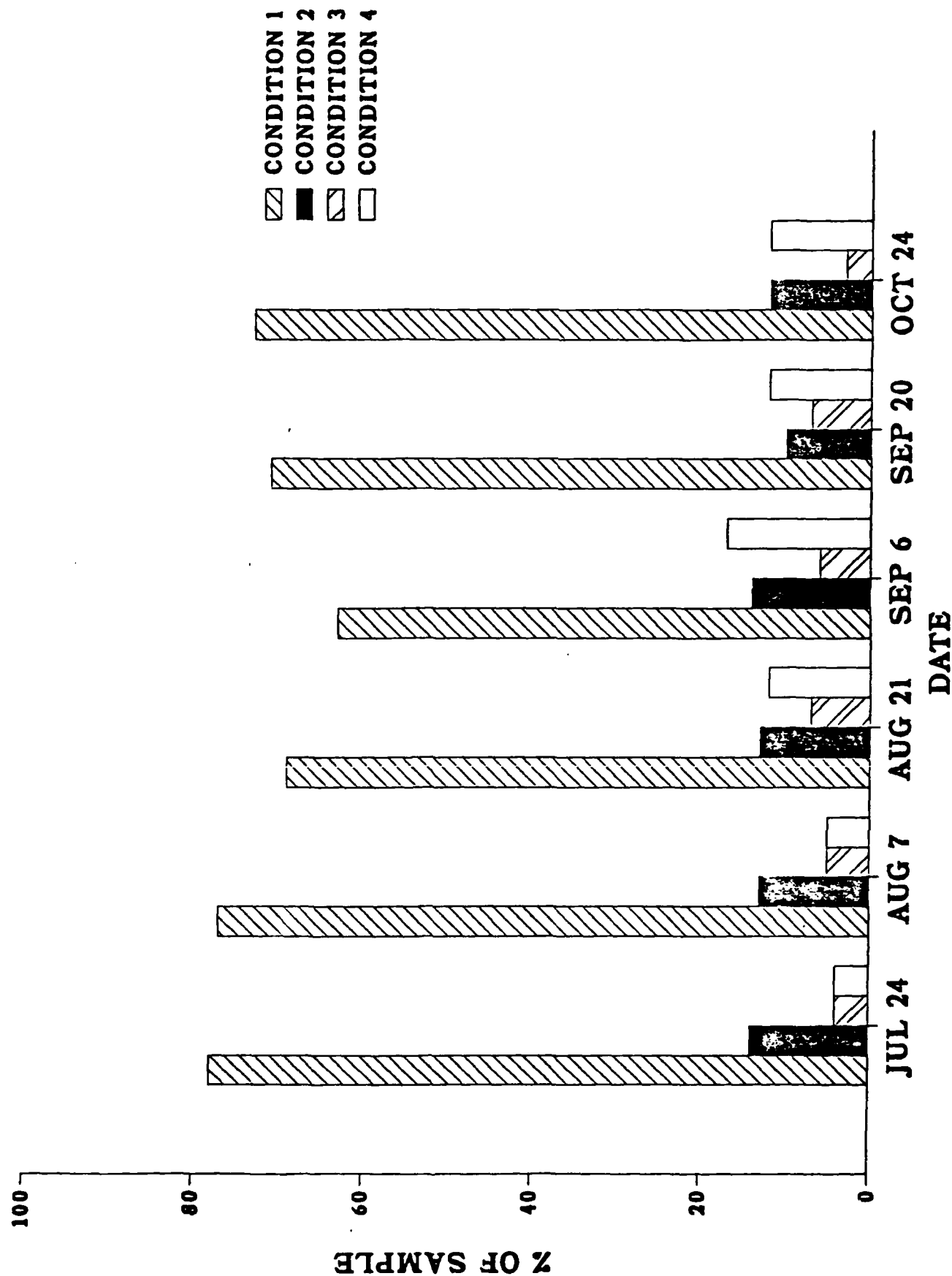
Comparisons made between individual plots within each site for various sampling dates showed no differences at the 0.05 α level of significance. Hence, reference will be made to the three study sites rather than individual plots.

A summary of information for each seedling sampling date is shown in Appendix J. Average seedling condition was relatively stable throughout this first season, with nonsignificant fluctuations among sites between 1.2 and 2.0. Figure 7 shows between 65 to 75% of the seedlings to be in seedling condition 1 throughout the summer.

Average plant moisture stress increased with time as shown in figure 8. Values at planting averaged -1.2 bars for all sites and increased to -20.0 bars for the antenna and ground site, and -11.0 bars at the control site by the late October. Differences among sites in October were largely related to the proportion of seedlings in each seedling condition class for the site. Figure 9 shows the relationship of plant moisture stress and seedling condition. While plant moisture stress increased with time for seedlings in condition 1, values were lower than for conditions 2, 3, and 4. Thus a site with greater numbers of seedlings in the latter classes would have a higher PMS value while the healthiest seedlings on the site have fairly uniform values. In addition, little variability was evident among sites for condition 1 seedlings before the beginning of dormancy (approximately the end of August) (Figure 10). The significance greater difference in PMS values among sites in the dormant season are unclear at this time.

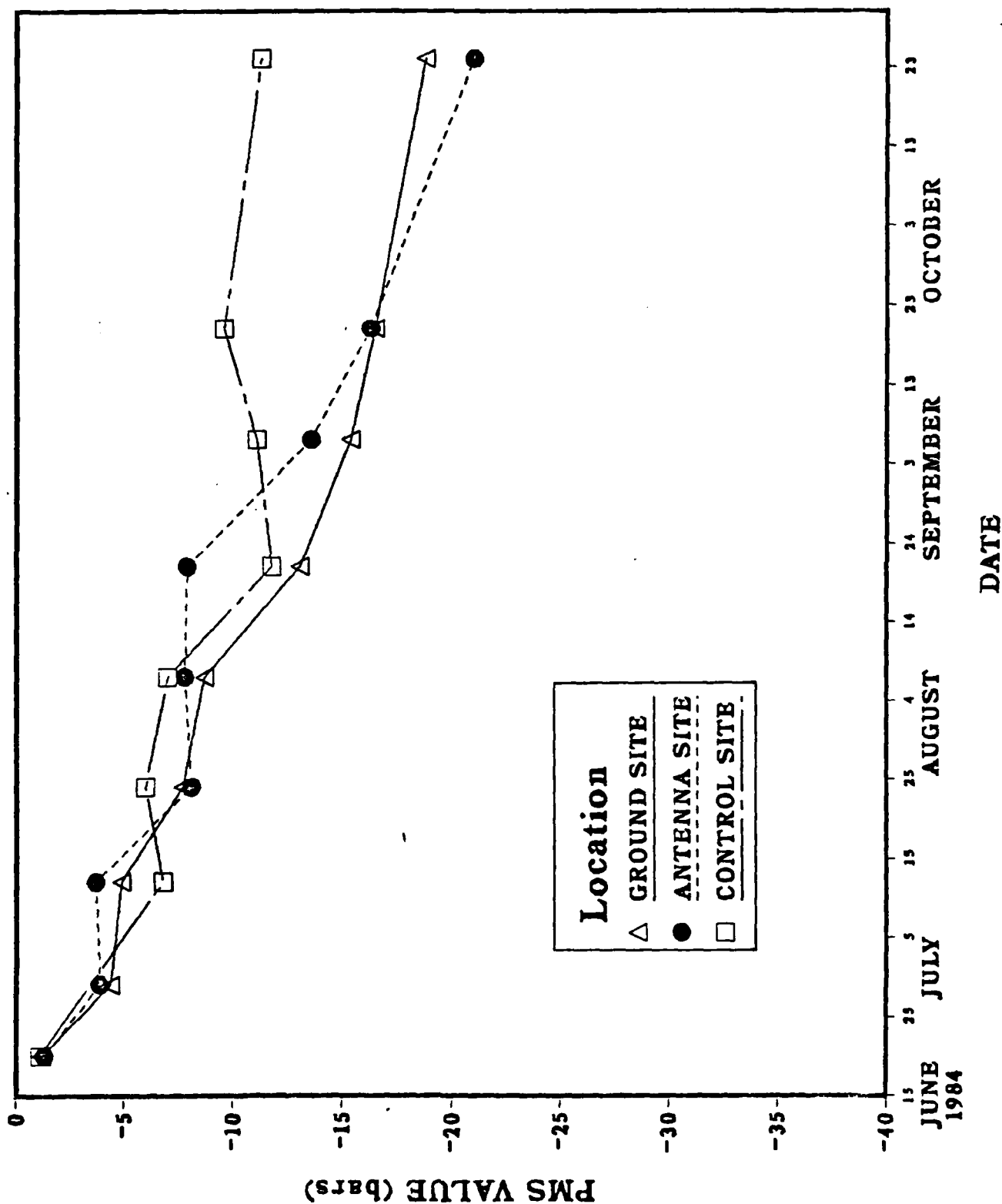
We expect that many of the seedlings with high proportions of brown needles (conditions 3 and 4) are or soon will be dead. These seedlings will be accounted for in survival estimates but will no longer warrant inclusion in the plant moisture stress and seedling growth studies. Plant moisture stress will be used as a covariate along with other ambient data to partition the variation in growth due to environmental variables from that possibly due to ELF fields.

FIGURE 7. PERCENTAGE OF SEEDLINGSBy Seedling Condition



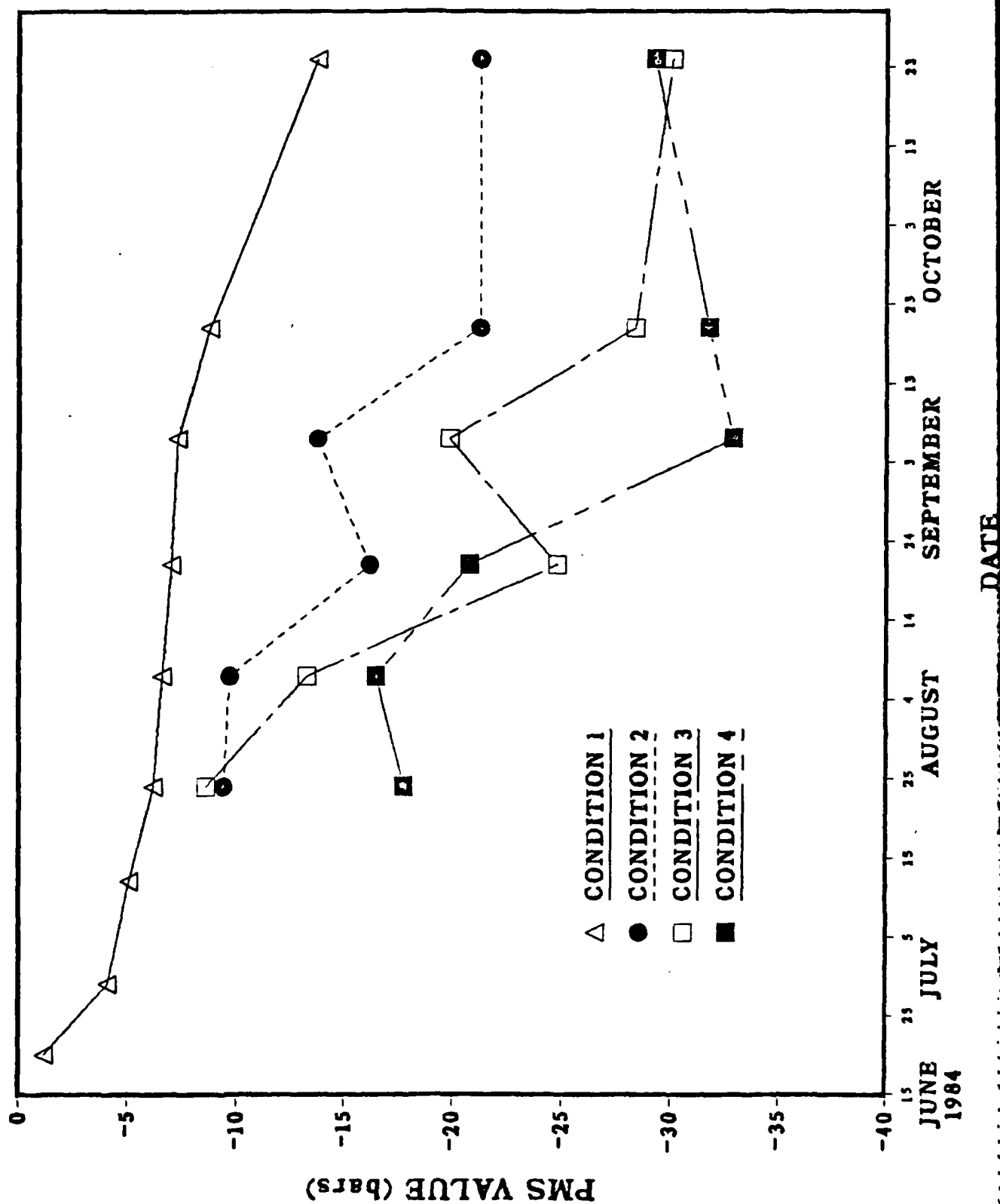
AVERAGE PMS VALUES **Site by Sampling Date**

FIGURE 8.



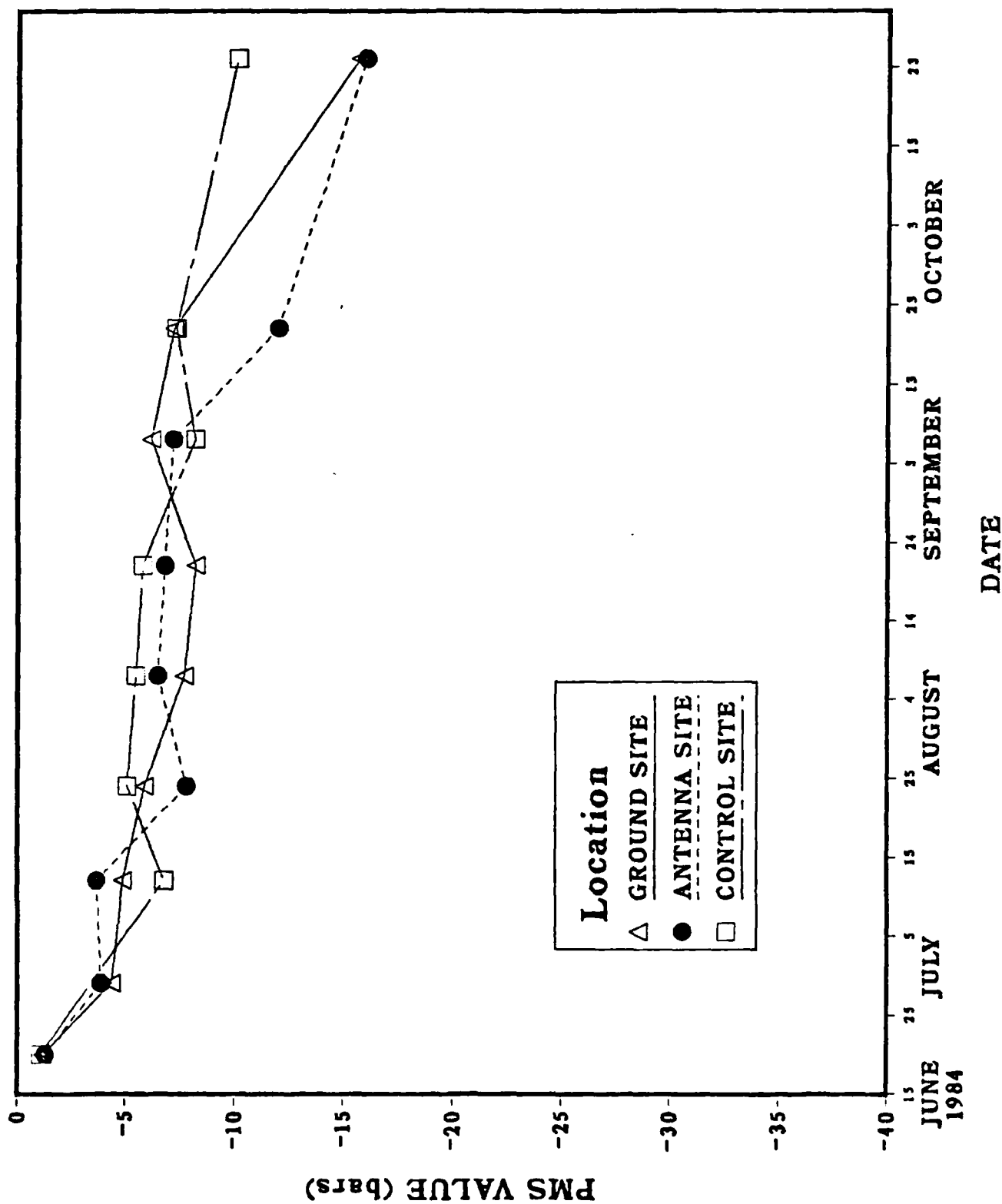
AVERAGE PMS VALUES By Seedling Condition

FIGURE 9.



AVERAGE PMS VALUES **Seedling Condition 1**

FIGURE 10.



Seedling growth

Three hundred red pine seedlings were permanently marked at the ground, antenna, and control sites and were measured at the end of the 1984 growing season. The following information was recorded from each seedling:

- Basal diameter (cm)
- Height to top of candle or bud (cm)
- Condition - percent of red needles, where
 - 1 = 0 - 25%
 - 2 = 25 - 50%
 - 3 = 50 - 75%
 - 4 = 75 - 100%
- Presence or absence of full bud formation (Bud set)

Seedling basal diameter was not different ($\alpha = 0.05$ level) among sites at the end of the growing season (Table 16). Diameters were .45 cm, .44 cm, and .46 cm for the ground, antenna, and control sites respectively. Seedling height was similar at the ground (16.8 cm) and antenna (16.6 cm) and only slightly greater at the control site (17.7 cm) (Table 17). A subsample of seedlings at the time of planting showed a mean diameter of .42 cm ($n=105$). However height measurements taken at the time of planting are not comparable with measurements taken at the end of the growing season because of varying planting depth. Future measurements taken to the top of the terminal bud each fall will be used together with diameter to compare growth among sites.

Error estimates varied by generally less than 3% for diameter and height on all sites (Table 16 & 17). The number of seedlings measured in

Table 16. Average diameter of red pine seedlings at the end of the first growing season.

Site	<u>Diameter</u>			
	Mean (cm)	Variance	n	Bounds of Estimation (cm)
Ground	.45	.0152	300	.0139
Antenna	.44	.0097	300	.0011
Control	.46	.0094	299	.0110

Estimated
Sample Size*% of⁺
Mean

29

19

17

+ Percent of mean within which estimation is possible.

* Sample size calculated to maintain a bound within 10% of the mean.

Table 17. Average height of red pine seedlings at the end of the first growing season.

Site	<u>Height</u>			
	Mean (cm)	Variance	n	Bounds of Estimation (cm)
Ground	16.8	21.30	300	.52
Antenna	16.6	15.86	300	.45
Control	17.7	17.94	299	.48

Calculated*
17% of⁺
Mean

29

22

22

+ Percent of mean within which estimation is possible.

* Sample size calculated to maintain a bound within 10% of the mean.

1984 to detect differences within 10% of the mean was more than adequate. These low error estimates reflect the genetic uniformity which is characteristic of red pine. However, the sample size will remain large for several years as environmental factors such as microsite may increase variation in diameter and height growth in subsequent years.

Percentages of seedlings within each condition class are presented in Table 18. The majority of seedlings on all sites were in condition class 1 with higher numbers occurring at the control. These estimates taken in late November are considerably lower than those for the last PMS sampling date in October (Figure 7), and may be due to a natural change in red pine needle color which occurs in late fall. The number of seedlings in condition class 4 is lowest at the control site and highest at the ground site. No significant differences ($\alpha = .05$) were found between mean diameter or mean height and condition class among the sites.

Percentage of seedlings with bud set within each condition class at the end of the 1984 growing season are shown in Table 19. Bud set is an indicator of "hardening" of the seedling before the beginning of the dormant season. The vast majority of seedlings in condition class 1 at each site set buds while approximately 1/2 of the seedlings set buds on condition class 2 seedlings. Only a few individuals set buds in condition classes 3 and 4. The control site recorded higher percentages of bud set in condition classes 1 and 2 than did the ground and antenna sites. There was no correlation between average diameter or height and occurrence of bud set among sites. Assuming 100% survival of all seedlings setting buds in condition class 1 and 2, we project a minimum of 3800 seedlings surviving per site. This sample size is more than adequate for future studies.

Table 18. Percentage of seedlings by condition class.

Condition Class	<u>Site</u>		
	Ground	Antenna	Control
1	52	54	61
2	16	20	19
3	6	10	9
4	26	16	11

Table 19. Percentage of seedlings with buds set within each condition class.

Conditon Class	<u>Site</u>		
	Ground	Antenna	Control
1	88	86	95
2	55	41	61
3	0	25	4
4	0	2	6

Detailed growth estimates will not be calculated this year because of the shock that most seedlings sustain in the planting process. However, these data will be used to develop techniques for analysis starting next year which will be similar to those outlined pole sized stands with the exception the PMS values will be included as a covariate in the seedling studies.

ELEMENT 4. PHENOPHASE DOCUMENTATION

Trees

The objective of this element is to describe and document various phenological events on selected tree species that would be used to indicate possible changes in physiological processes due to ELF electromagnetic fields. During the baseline study period efforts are being made to describe, quantify and document bud burst, leaf out, flowering, cambial activity, seed dissemination, and leaf fall of northern red oak, paper birch, bigtooth aspen and red maple. Candle elongation and bud burst will also be measured on red pine seedlings.

During 1983 phenophases of hardwood species were documented by compiling a photographic record of key biological events. Since phenological observations are generally subjective in nature, photographs must provide precise descriptions of specific developmental stages that would be used as standards to insure uniformity in year to year measurements. However, it was found that specific stages of bud burst, leaf out, and flowering were difficult to document using the photographic method. These events appeared different depending where on the tree the observation was made. Differences in bud burst and leaf out were recorded between lower branches and those higher in the canopy which receive a higher incidence of direct sunlight. Obtaining photographs from the upper portion of the tree canopy was also difficult. In addition, the timing of these events varied tremendously within individual trees and among species. This was particularly evident in bigtooth aspen where clonal differences were expressed.

Litter traps were used in an attempt to document flowering and seed dissemination of hardwood species. As flowers and seeds matured, they fell into the traps and weights obtained. However, these tree components

partially disintegrate as they mature, making positive identification and collection nearly impossible. The time required to empty traps and process samples at intervals necessary to adequately document these phenophases was also prohibitive. Because the identification of many small flower and seed parts is difficult, our ability to confidently detect differences from year to year is questionable. Consequently we recommend that the detailed study of these events be discontinued.

Leaf fall timing of hardwood species was documented by using 15 1 m² litter traps at the antenna and control sites. Litter was collected monthly during the summer growing season and weekly during the leaf fall period. Samples were separated into leaf, wood, and miscellaneous (seeds, flowers etc.) components, dried at 60°C in a forced air oven, and weighed. On each sample date leaf litter from three traps per site were separated by species. A summary of leaf fall percentage by species and time appears in Table 20. Northern red oak retained its leaves longer than other species, with 83% and 88% occurring during the last week of the leaf fall at the antenna and control site respectively. Red maple exhibited site differences losing 77% of its leaves during periods 2 and 3 at the antenna site, while at the control 73% fell in period 4. A similar leaf fall pattern was found with bigtooth aspen. In contrast, leaf fall of paper birch/hazelnut occurred faster at the control site than at the antenna site.

Error estimates for mean litter weight were very high for all species at each site. Calculated sample sizes necessary to detect differences within 10% of the mean are prohibitive in terms of time and effort required for collection and processing. This is true for both litter weight for each species at each sampling date, and cumulative seasonal totals. These variable results were due in part to collecting only three samples/site at each date. Sampling of all 15 litter traps at each site

Table 20. Percent of total leaf fall (by weight) for each sampling time period by site and species - 1984.

PERIOD	DATE	RED MAPLE		NORTHERN RED OAK		BIG TOOTH ASPEN		BIRCH/HAZELNUT*	
		ANTENNA	CONTROL	ANTENNA	CONTROL	ANTENNA	CONTROL	ANTENNA	CONTROL
1	7/19-8/29	0.5	4.1	0.6	0.9	0.9	0.3	0	3.0
2	8/29-10/3	23.7	6.2	12.9	8.3	34.4	16.0	25.7	60.1
3	10/3-10/10	53.0	16.5	3.7	2.8	49.8	31.0	69.1	29.5
4	10/10-10/17	22.8	73.2	82.8	87.8	14.9	52.6	5.2	7.5
5	10/17-10/24	0	0	0	0	0	0	0	0

* Paper birch and hazel nut leaves are indistinguishable after collection and thus were combined.

would be necessary to obtain reasonable error estimates and confidence intervals for each tree species.

The onset and termination of cambial activity was determined from weekly readings of permanently installed dendrometer bands placed on all trees 10 cm and larger at both the antenna and control site (See Element 3 - Tree Productivity). However, because of dendrometer band inaccuracy for a short period following initial installation, the dendrometer bands could not detect onset of cambial activity this first year. However, termination of diameter growth was determined and is expressed as the number of trees whose growth stopped at a given date (Table 21). Generally, growth was completed for all species at the control site between September 6 through September 20. At the antenna site growth stopped between September 20 and October 3 for northern red oak and bigtooth aspen, while paper birch and red maple showed patterns similar to the control site. As would be expected, the halting of diameter growth immediately preceded the major leaf fall (See Element 8 - Litter Production). Trees in the smaller diameter classes showed a tendency to terminate diameter growth at an earlier date. These small diameter trees tended to be overtopped by larger trees and were in a suppressed condition. Further investigation of growth termination will be determined by time series analysis which will incorporate ambient data as a factor. This work will continue in 1985.

Table 21. DATE OF DIAMETER GROWTH TERMINATION

Antenna Site

Species/Diameter Class (cm)	DATE												
	7/19	7/26	8/2	8/9	8/16	8/23	8/29	9/6	9/13	9/20	10/3	10/17	n
Northern Red Oak													
10-14			1	1			2	1	1	3	1		10
15-19					1			1	4	1	3		10
20-24	1			2		1	2		1	3	3		13
25-30 ⁺								1		1	1		3
31-34										2	1		3
Paper Birch													
10-14				1					1				2
15-19						1		2					3
20-24				1									1
25-30 ⁺							2						2
31-34													0
Bigtooth Aspen													
10-14											1		1
15-19													0
20-24							1			3			4
25-30 ⁺	1								1	4	3		9
31-34										1			1
Red Maple													
10-14						3	3	9	6	11	5		44
15-19			2	5		4	2	8	9	12	5		46
20-24			2	2	2		1	4	2	3			10
25-30 ⁺									3				3
31-34						1				2			3

Table 21 CONT'D. DATE OF DIAMETER GROWTH TERMINATION

[illegible]

Because of the late planting date, many red pine seedlings had broken dormancy while in storage making the measurement of spring phenological events impossible. Bud set observations were made and are reported in element 3 but no temporal relationships were attempted this first year due to normal seedling planting shock and associated mortality. This coming spring, a regular schedule of phenological observations will be made starting with bud burst and candle elongation as previously planned.

Herbaceous Plants

The herbaceous layer, of a northern hardwood ecosystem, is an ecologically important component of the system with respect to edaphic and vegetative factors. Phenology, the study of the timing of life cycle events relative to environmental cues (Barbour et al 1980), has been used to quantitatively describe the herbaceous component of a northern hardwood forest (Mahall and Bormann 1978). Many herbaceous studies focused on phenology revolve around the observation and measurement of reproductive organs, i.e. flowering parts, as well as vegetative organs, i.e. leaf parts. Our approach thusfar has been to concentrate observations on the period of flowering and fruiting.

Forty permanent meter square quadrats were established at each site for Trientalis borealis, Aster macrophyllus, Anemone quinquefolia, and Pteridium aquilinum. Relative cover was estimated per meter square quadrat for each herb species within a quadrat. This data, to be compared from year to year on a per quadrat basis, was collected to illustrate changes in cover values for an herb other than the one chosen for phenological observation. The period of flowering and fruiting at both sites lasted from late May through mid August. However, the number of plants actually producing flowers and therefore available for study, was low. For example only 38%

of the Trientalis borealis at the control plot flowered while 44% at Martell's lake flowered. Of those that flowered, the numbers of 1 and 2 flowered plants were similar between sites (Table 22) showing a high degree of uniformity between sites.

Table 22. Proportion of 1 and 2 flower plants (Trientalis borealis) at the antenna and control sites.

<u>Site</u>	<u>One flower/plant</u>	<u>Two flowers/plant</u>
	—% of plants flowering—	
Control	27	11
Antenna	31	10

However, we feel that the number of individuals severely limits the statistical validity of this approach. While increasing the number of plots could improve the precision of this study, subsequent vandalism at the control site, which destroyed 38 of 40 one meter squared plots has dictated a closer look at the continuation of this work. The destroyed plots are impossible to relocate therefore most of the work done to date is of little use in future study. For these compelling reasons we propose a revised course of study.

The specific objective of this new course of study will be to quantify the timing of certain phenological events, on Trientalis borealis, on a continuous basis. Trientalis has been chosen for intensive study since our past work has indicated:

- 1) there are adequate numbers of individual plants on the plots for statistical sufficiency

- 2) *Trientalis* flowers most prolifically of all herbaceous plants on the plots
- 3) phenological events have been well defined and documented so that little additional development work will be necessary.

To obtain a sample size of approximately 200 individual plants per site, transect lines of variable length will be used. The line will be divided into subplots of 1 square meter to quantify the individuals found at each site. Parameters to be measured per plant on twice weekly visits are:

1. Emergence. Date noted on first visit.
2. Number of leaves.
3. Leaf measurement - length and width of largest leaf to determine leaf maturation (Brown et al. 1985).
4. Plant height - measured from root collar to leaf whorl to determine time to maximum height.
5. Flowering - both flower open and number of flowers will be recorded until calyx closure to determine the flowering period (Anderson et al. 1983).
6. Yellowing - the number of leaves with any yellowing will be recorded to determine the senescence period.
7. Death - the number of leaves completely brown will be recorded to determine the drying period.

Photosynthetically active radiation (PAR) has been chosen as a covariate of major interest since it has been shown to be a primary factor in controlling phenological events. In this study, if ELF fields have an effect on tree canopy, more or less light would reach the subcanopy vegetation. Thus effects on the canopy vegetation could have an effect on

the herbaceous layer. Other covariates include photoperiod and air temperature.

PAR was also chosen since it is a precise measure of radiation available to herbaceous plants. Photoperiod alone is considered inadequate since it is not a measure of radiation intensity. PAR will be quantified using Licor 1905 quantum sensors connected to the existing ambient monitoring system.

Covariate analyses will be used to test year to year differences in initiation and duration of emergence leaf maturation, flowering, senescence and death.

Element 5 Herbaceous Vegetation Cover

Since composition of herbaceous plant cover has been commonly found to be influenced by environmental changes, a primary measure of response is species diversity. This can be evaluated by using species presence, coverage and/or biomass. Our original approach included biomass, however last year's report showed the extreme variation associated with the model's prediction of plant component weights making the development of equations prohibitively expensive. This past year, we have concentrated on plant coverage to evaluate changes due to ELF field effects.

Progress

Because of the high scientist traffic on plots due to frequent dendrometer band readings, herbaceous reserve areas were established immediately adjacent to the trees plots. Within these reserves, 3 blocks were established with 3 - 35 meter long transects per block for a total of 9 transects per site. Relative coverage of each species on each transect was measured using the line intercept method described by Canfield (1941). Coverage measurements were made at the beginning of August.

Of the 14 species representing approximately 90% of the total relative cover, only 4 species were common to both sites (Table 24).

The presence/absence of herb species between the control and antenna sites was quantified on the reserve area transects using the Jaccard similarity index (Mueller-Dombois, 1974) Where:

$$IS_j = \frac{\text{Common Species}}{(\text{Species unique to one population}) + (\text{Species unique to the other population}) + (\text{Common Species})} \times 100$$

The calculated index was 32% showing only moderate similarity between the two sites. The Sorenson index, where:

$$IS_s = \frac{\text{Common species}}{\frac{1}{2}(\text{\#species in 1 population}) + (\text{\#species in another population})} \times 100$$

indicated greater similarity at 61%. Sorenson's index gives greater weight to species that are common to both areas rather than those that are unique which is more applicable to this analyses.

To monitor the developement of natural herbaceous communities in the plantation areas, transects, using the same design described earlier, were established adjacent to the seedling plots. These transects were measured by the line intercept method in mid-June and relative cover calculated. Thirteen (13) species comprise approximately 90% of the total relative cover with 5 species common to both sites (Table 25). Again, comparing the control and antenna sites for the plantation plots produced a Jaccard index value of 33% and a Sorenson index value of 65%. To further quantify herbaceous cover and species diversity, 4 30X61 cm plots (Total of 36 per site) will be established systematically along each transect on both the herb reserves and plantation plots. Frequency of occurence and coverage will then be determined at each site.

Analysis of frequency and cover will follow in a covarrate analysis as described for trees (Element 3). Replicates will result from the four 30X61 cm plots along the nine transects at each site. Factors in the ANOVA design will include site and time where the two sites are the control and antenna sites and time is considered at each year before and after the antenna is operational. Covariates such as site and climatological variables will be included to reduce random errors resulting from these

influences. Each year frequency and coverage data will be compared among sites and to data from previous years to establish a standard by which to compare ELF field effects.

Table 24. Species comprising 90% of relative cover on herbaceous plant reserves.

Species	-----Relative Cover % -----	
	Antenna Site	Control Site
<i>Arelia nudicaulis</i>	---	---
<i>Anemone quinquefolia</i>	1.7	---
<i>Asteri macrophyllus</i>	4.2	5.6
<i>Gaultheria procumbens</i>	5.9	1.8
<i>Mianthemum Canadense</i>	---	3.7
<i>Lycopodium Clavatum</i>	1.7	---
<i>Pteridium aquilinum</i>	59.5	46.2
<i>Rubus allegheniensis</i>	2.6	---
<i>Rubus idaeus</i>	---	4.5
<i>Rubus parviflorus</i>	4.0	---
<i>Trientalis borealis</i>	---	7.9
<i>Vaccinium membranaceum</i>	4.2	3.3
<i>Viburnum acerfolium</i>	---	11.1
<i>Waldsteinia fragaria</i>	---	1.6

Table 25. Species comprising 90% of relative cover on plantation sites prior to timber harvest.

Species	----- Relative Cover % -----	
	Antenna Site	Control Site
Aster macrophyllus	2.1	35.9
Gaultheria procumbens	4.4	---
Lycopodium obscurum	---	2.7
Mianthemum canadense	1.9	3.2
Oryzopsis asperifolia	2.9	1.3
Pteridium aquilinum	50.8	17.1
Rubus allegheniesis	---	2.2
Rubus idaeus	---	2.2
Pubus parviflorus	16.2	---
Trientalis borealis	7.0	---
Trillium grandiflorum	---	1.6
Vaccinium membranacem	5.6	2.2
viburnum acerifolium	---	21.4

ELEMENT 6. Mycorrhizal Fungi Collection

Mycorrhizae represent finely balanced physiological relationships between the roots of higher plants and a number of highly specialized and beneficial fungi. Mycorrhizal fungi parasitize the fine roots of their hosts, utilizing host photosynthate as energy with which to forage for minerals and water in the forest floor. The matrix of mycorrhizal mycelium permeating the soil from infected roots provides the host with scarce minerals and water much more efficiently than could the host's roots alone.

Ectomycorrhizae are one of the two major types of mycorrhizae which form on forest trees. They represent an ideal system for detection of environmental perturbations in forest ecosystems for several reasons. Ectotrophic mycorrhizae lend themselves readily to extensive population dynamics study. Ectotrophic fungi produce a mantle which affects feeder root development, resulting in more or less characteristic rootlet morphology patterns. Also, ectotrophic fungi characteristically produce relatively large and identifiable fruiting bodies (sporocarps) which aid greatly in population studies. Furthermore, many ectotrophic fungi can be isolated into pure culture from fruiting bodies or surface-sterilized mycorrhizal feeder roots. Finally, mycorrhiza population dynamics reflect the influence of perturbations on both host and parasite in such a way that effects on either the host or the parasite component may be reflected by effects on the other component. This relationship may prove valuable in corroborating evidence of ELF effects which might develop in other work elements of this research program.

The objectives of this work element are 1) to develop reference collections of cultures and freeze-dried sporocarps representing as many as possible of the presumed mycorrhizal fungus species occurring at the study

sites, and 2) to characterize the population dynamics of sporocarp production for these fungi in the study plantations and pole-stands.

The reference collections serve two purposes. First, mycorrhizal fungi isolated into pure culture from roots as part of Element 7 can only be identified by comparison of their cultural growth characteristics with those of known species isolated into pure culture from identified sporocarps. Second, the reference sporocarp collection serves to vouch for the identity of the species included in the sporocarp population dynamics component of this element. Many of these species cannot be isolated into pure culture, and are therefore only identifiable by microscopic examination of sporocarps.

Population dynamics of sporocarp production is being studied by periodic monitoring of sporocarp production on the three contiguous 1050 square meter herbaceous reserve plots located within the overhead antenna and control sites. The herbaceous reserve plots represent the pole-stands typical of each study site. The large study plot size should minimize the variability of sporocarp counts between years by absorbing the effect of annual spatial redistribution of sporophore production around host trees. Sporocarp production is closely tied to host photosynthetic activity (Last et al. 1979), and can be expected to proceed as regularly as relatively stable forest stands and climate will permit. Sporocarp production will be monitored in the red pine plantations as well. Periodic monitoring of fruiting over an extended period each year is necessary because fruiting of mycorrhizal fungus species differs phenologically and is relatively ephemeral. Ambient monitoring data will be used to account for the influence of moisture and temperature on fruiting over time at the different

study sites. Techniques for characterizing and comparing fungal populations via fruiting body production have been published (Grainger 1946, Parker-Rhodes 1951, Hering 1966, Richardson 1970, Fogel 1976, Fogel 1981). Other quantitative concepts which may prove useful in comparing data sets include coefficient of community (Pielou 1977), and Orloci's sums of squares method based on standardized distances (Orloci 1967). Similarities in overstory species composition of ectomycorrhizal hosts between the study sites support the presumption that mycorrhizal populations at the study sites should be reasonably well-matched. The quantitative techniques to be used, however, do not require that sites be perfectly matched initially in order to detect relative population changes which develop over time experimentally. Any effects of ELF fields on the fruiting dynamics of mycorrhizal fungi will be detected as shifts in the relative representation of fruiting species at each site over a period of years.

Progress

Sporocarps of ectomycorrhizal fungi occurring on the 30 m X 35 m plots were inventoried at 1 to 2 week intervals between early August and mid-October, 1984. Each plot was divided into four strips 7.5 m wide to facilitate survey. Each sporocarp tallied was slit across the pileus with a sharp knife to prevent it from being tallied more than once. In order to estimate the longevity of sporocarps in the field, 343 specimens representing 33 species were flagged during the fall of 1984 for repeated observation. In this way, the likelihood of under-representing certain species through weekly or bi-weekly visitation intervals can be estimated.

When possible, specimens were collected for freeze-drying and for isolation attempts. In general, tallied specimens were left on the plots to sporulate. The slit pileus was often the only mark of the survey. All cultural isolation attempts from fruiting bodies were made by plating small portions of stipe or pileus context tissue onto modified Melin-Norkrans agar medium (Molina and Palmer 1982), the same medium used for isolation attempts from mycorrhizal root tips (Element 7). Between 10 and 20 isolations were attempted from each selected specimen.

It is apparent from the 1984 survey data that mycorrhiza populations on the study sites, and especially in the hardwood pole-stands, should be monitored via sporocarp production. This method has the advantages of including fungal species which cannot be isolated into pure culture from roots as well as fungal species associated with tree species other than red pine. Data collected in 1984 suggest that sufficient fruiting by a number of mycorrhizal species does take place to permit detection of any meaningful population disturbance.

Table 26 summarizes the seasonal production of sporocarps by 38 presumed mycorrhizal fungi. Only 15 of these species were found in the first survey on 4 August, while only 4 species were encountered during the last survey on 13 October. Only 4 species resulted in sporocarp frequency modes coinciding with the first visit, and none demonstrated a mode in October. This agrees well with the facts that litter fall on both sites was nearly complete by 17 October (Element 8) and that the first frost on both sites occurred in late September. Mycorrhizal fruiting has been shown to be sensitive to declining photosynthesis in the host plants (Last, et al. 1979). The data suggest that August and September are the months of peak

Table 26. Seasonal distribution of fruiting by presumed mycorrhizal fungi on herbaceous reserve plots (1050 square meters each) at the overhead antenna and control sites between 4 August and 13 October, 1984.

Family	Genus	Species	Earliest Record		Latest Record		Weighted Midpoint ^b		Mode ^c	
			Antenna	Control	Antenna	Control	Antenna	Control	Antenna	Control
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u>	1 Sept.	1 Sept.	30 Sept.	13 Oct.	1 Sept.	1 Sept.	1 Sept.	4 Aug.
		<u>brunneascens</u>	4 Aug.	4 Aug.	30 Sept.	22 Sept.	18 Aug.	4 Aug.	30 Sept.	22 Sept.
		<u>citrina</u>	1 Sept.	1 Sept.	13 Oct.	30 Sept.	30 Sept.	30 Sept.	1 Sept.	---
Boletaceae	<u>Ananitopsis</u>	<u>muscaria</u>	4 Aug.	---	16 Sept.	---	1 Sept.	---	18 Aug.	18 Aug.
		<u>vaginata</u>	4 Aug.	---	18 Aug.	---	18 Aug.	---	---	---
		<u>chrysenteroides</u>	---	18 Aug.	---	18 Aug.	---	---	---	---
Cantharellaceae	<u>Boletus</u>	<u>rupestris</u>	---	18 Aug.	---	1 Sept.	---	---	---	---
		<u>piperatus</u>	18 Aug.	16 Sept.	16 Sept.	16 Sept.	1 Sept.	1 Sept.	1 Sept.	16 Sept.
		<u>scabrum</u>	18 Aug.	1 Sept.	16 Sept.	22 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
Cortinariaceae	<u>Cantharellus</u>	<u>lutescens</u>	---	4 Aug.	---	22 Sept.	---	---	---	---
		<u>albobolaceus</u>	1 Sept.	16 Sept.	30 Sept.	30 Sept.	16 Sept.	22 Sept.	16 Sept.	18 Aug.
		<u>armillatus</u>	18 Aug.	18 Aug.	30 Sept.	13 Oct.	1 Sept.	1 Sept.	1 Sept.	22 Sept.
Elaphomycetaceae	<u>Rozites</u>	<u>flavifolius</u>	18 Aug.	18 Aug.	1 Sept.	1 Sept.	1 Sept.	1 Sept.	1 Sept.	18 Aug.
		<u>semisanguineus</u>	16 Sept.	16 Sept.	16 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
		<u>sphaerosporus</u>	16 Sept.	16 Sept.	30 Sept.	22 Sept.	16 Sept.	16 Sept.	16 Sept.	22 Sept.
Hydnaceae	<u>Elaphomyces</u>	<u>trivialis</u>	1 Sept.	16 Sept.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	22 Sept.
		<u>capitata</u>	18 Aug.	18 Aug.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	22 Sept.
		<u>granulatus</u> ^a	18 Aug.	18 Aug.	13 Oct.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
Rhodophyllaceae	<u>Dentinum</u>	<u>repandum</u>	---	4 Aug.	---	30 Sept.	---	---	---	---
		<u>zonatum</u>	---	16 Sept.	---	16 Sept.	---	---	---	---
		<u>spp.</u>	4 Aug.	18 Aug.	16 Sept.	30 Sept.	1 Sept.	16 Sept.	16 Sept.	16 Sept.
Russiaceae	<u>Rhodophyllus</u>	<u>argillaceifolius</u>	4 Aug.	16 Sept.	30 Sept.	16 Sept.	1 Sept.	16 Sept.	16 Sept.	16 Sept.
		<u>argillaceifolius</u>	1 Sept.	18 Aug.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
		<u>rufus</u>	18 Aug.	4 Aug.	16 Sept.	22 Sept.	1 Sept.	1 Sept.	1 Sept.	1 Sept.
Russiaceae	<u>Russula</u>	<u>subvellerus</u>	18 Aug.	16 Sept.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	22 Sept.
		<u>tormentosus</u>	16 Sept.	16 Sept.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	22 Sept.
		<u>brevipes</u>	4 Aug.	4 Aug.	18 Aug.	22 Sept.	4 Aug.	18 Aug.	18 Aug.	18 Aug.
Tricholomataceae	<u>Cantharellula</u>	<u>emetica</u>	4 Aug.	4 Aug.	30 Sept.	22 Sept.	1 Sept.	4 Aug.	1 Sept.	4 Aug.
		<u>fragilis</u>	18 Aug.	18 Aug.	30 Sept.	22 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
		<u>laurocerasi</u>	4 Aug.	4 Aug.	1 Sept.	22 Sept.	18 Aug.	18 Aug.	18 Aug.	18 Aug.
Tricholomataceae	<u>Clitocybe</u>	<u>paludosa</u>	18 Aug.	18 Aug.	16 Sept.	22 Sept.	18 Aug.	18 Aug.	18 Aug.	18 Aug.
		<u>subdepallens</u>	18 Aug.	4 Aug.	30 Sept.	22 Sept.	18 Aug.	18 Aug.	18 Aug.	18 Aug.
		<u>variata</u>	4 Aug.	4 Aug.	30 Sept.	30 Sept.	1 Sept.	1 Sept.	1 Sept.	4 Aug.
Tricholomataceae	<u>xerampelina</u>	<u>umbonata</u>	18 Aug.	1 Sept.	1 Sept.	16 Sept.	---	---	---	---
		<u>gibba</u>	---	1 Sept.	---	16 Sept.	---	---	---	---
		<u>laccata</u>	4 Aug.	4 Aug.	30 Sept.	30 Sept.	18 Aug.	18 Aug.	18 Aug.	16 Sept.
Tricholomataceae	<u>Tricholoma</u>	<u>flavovirens</u>	18 Aug.	4 Aug.	30 Sept.	30 Sept.	16 Sept.	16 Sept.	16 Sept.	16 Sept.
		<u>resplendens</u>	30 Sept.	---	30 Sept.	---	30 Sept.	---	---	---
		<u>resplendens</u>	30 Sept.	16 Sept.	30 Sept.	30 Sept.	30 Sept.	22 Sept.	30 Sept.	30 Sept.

a. Presence detected by association with fruiting of *Cordyceps* spp.
 b. Date by which 50 percent of the season's fruiting had taken place.
 c. Date of most abundant fruiting.
 d. The mode is shared more than 1 date.

fruiting by most of the mycorrhizal fungus species observed to fruit on the study sites. One additional sampling in late July might clarify the seasonality of fruiting by the four fungi with 4 August population modes (Amanita brunnescens, Russula emetica R. laurocerasi, and R. xerampelina).

Table 27 summarizes the data on longevity collected in 1984. The number of flagged specimens representing each species is largely a function of specimen availability and condition on survey dates. These data help to identify those species for which weekly or bi-weekly surveys will most precisely characterize sporocarp population dynamics. Species for which sporocarps do not often survive for one week must be markedly under-represented and would have to be relatively prolific producers of sporocarps in order to provide useful data. Additional longevity data will be collected in 1985. The 1984 and 1985 data will be evaluated together to aid the identification of key species.

Table 28 summarizes the spatial distribution of sporocarp production over the three plots each at the overhead antenna and at the control site. In general, 36 species are known to occur at both sites. Additional species are well-represented at one site only. Large differences occur in the apparent distributions of individual species between plots at one or both sites. Population differences between plots and sites will present little difficulty for purposes of data analysis. These differences reflect site and stand characteristics rather than the intrinsic variability in annual fruiting within plots. We presume, for the present time, that plot size is large enough (1050 sq. m.) to account for much of the annual variation in fruiting intensity by the fungus species studied. Ambient monitoring data for 1985 should permit a more objective comparison of the study plots and sites.

Table 27. Longevity in the field of flagged fruiting bodies of presumed mycorrhizal fungi.

Family	Genus	Species	Number of Specimens Flagged	Longevity ^a (weeks)			
				1	2	3	4
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u>	8	5			
		<u>brunnescens</u>	3	0			
		<u>citrina</u>	15	2			
		<u>muscaria</u>	13	4	1		
		<u>fulva</u>	2	0			
Boletaceae	<u>Amanitopsis</u> <u>Boletus</u>	<u>piperatus</u>	1	0			
		<u>scabrum</u>	7	5			
		<u>tutescens</u>	1	0	1		
		<u>alboviolaceus</u>	37	21	12		
		<u>armillatus</u>	26	7	13	3	
Cantharellaceae	<u>Cantharellus</u>	<u>flavifolius</u>	13	3	9		
		<u>semisanguineus</u>	6	1	1		
		<u>trivialis</u>	21	14			
		<u>caperata</u>	34	15	3		
		<u>repandum</u>	7	1	3	1	2
Hydnaceae	<u>Rozites</u> <u>Dentinum</u>	<u>spp.</u>	7	5			
		<u>argillaceifolius</u>	9	2	3	2	
		<u>piperatus</u>	1	1			
		<u>rufus</u>	14	7			
		<u>subvellerus</u>	15	6	6	2	
Rhodophyllaceae	<u>Rhodophyllus</u>	<u>terminosus</u>	12	8	1		
		<u>uvidus</u>	1	1			
		<u>brevipes</u>	2	2			
		<u>emetica</u>	15	4			
		<u>paludosa</u>	1	0			
Russulaceae	<u>Russula</u>	<u>subdepallens</u>	8	2			
		<u>variata</u>	23	8	1		
		<u>gibba</u>	4	3	1		
		<u>odora</u>	10	8	2		
		<u>laccata</u>	14	5	9		
Tricholomataceae	<u>Clitocybe</u> <u>Laccaria</u> <u>Tricholoma</u>	<u>flavovirens</u>	3	1			
		<u>resplendens</u>	5	3			

a. Number of specimens surviving in identifiable conditions; specimens are only reported once under the maximum age attained in identifiable condition.

Table 28. Spatial distribution of presumed mycorrhizal fungus fruiting bodies between herbaceous reserve plots (1050 square meters each) at the overhead antenna and control sites between 4 August and 13 October, 1984.

Family	Genus	Species	Overhead Antenna				Control			
			Plot 1	Plot 2	Plot 3	Total	Plot 1	Plot 2	Plot 3	Total
Amanitaceae	<i>Amanita</i>	<i>floridigera</i>	6	15	1	22	1	1	0	2
		<i>brunneocens</i>	1	9	30	40	1	4	17	22
		<i>clitinea</i>	3	6	9	18	1	3	4	8
		<i>muscaria</i>	12	33	7	52	0	0	0	0
Boletaceae	<i>Amantopsis</i>	<i>virginata</i>	1	1	1	3	1	5	1	7
		<i>trifida</i>	0	6	0	6	0	0	0	0
	<i>Imanella</i>	<i>chrysotheloides</i>	0	0	0	0	0	0	0	0
		<i>russetii</i>	0	0	0	0	12	0	12	21
	<i>Boletus</i>	<i>piperatus</i>	4	9	4	17	0	0	1	1
		<i>subglabripes</i>	0	0	0	0	0	0	0	0
	<i>Leccinum</i>	<i>glabrum</i>	3	1	0	4	0	16	0	20
		<i>chromapes</i>	0	0	0	0	2	1	0	3
	<i>Cantharellus</i>	<i>infestans</i>	0	0	0	0	0	0	0	0
		<i>cornucopioides</i>	0	0	0	0	35	20	44	99
Cortinariaceae	<i>Cortinarius</i>	<i>alberici</i>	3	114	1	118	0	0	1	1
		<i>armillatus</i>	6	4	6	16	4	32	65	102
	<i>Hebeloma</i>	<i>pholideus</i>	0	0	0	0	1	1	11	13
		<i>sangolius</i>	1	1	0	2	0	0	0	2
	<i>Rozites</i>	<i>capitata</i>	43	52	0	95	0	0	2	97
		<i>sp.</i>	36	69	0	105	0	0	5	110
	<i>Elaphomyces</i>	<i>glaucocephalus</i>	31	22	11	64	29	11	11	51
		<i>rutillus</i>	9	12	11	32	2	55	22	79
	<i>Gomphidiales</i>	<i>repandum</i>	0	0	0	0	0	0	0	0
		<i>torquatum</i>	0	0	0	0	0	0	0	0
Hydnaceae	<i>Hydnophorus</i>	<i>aurantiacus</i>	0	0	0	0	0	0	0	0
		<i>involutus</i>	0	0	0	0	0	0	0	0
	<i>Coltricia</i>	<i>perennis</i>	0	3	1	4	0	0	0	4
		<i>sp.</i>	39	49	26	114	19	43	32	94
	<i>Rhodophyllus</i>	<i>affinis</i>	0	0	0	0	0	0	0	0
		<i>lucifolius</i>	26	31	51	108	4	6	2	12
	<i>Lactarius</i>	<i>piperatus</i>	0	0	0	0	0	0	0	0
		<i>resinus</i>	0	0	0	0	0	0	0	0
	<i>Polyporeae</i>	<i>rufus</i>	32	484	302	898	1	70	0	71
		<i>subvellerus</i>	7	0	0	7	9	14	26	49
Russulaceae	<i>Russula</i>	<i>torinosus</i>	5	1	0	6	3	22	5	30
		<i>villosus</i>	0	1	0	1	0	0	0	0
	<i>Ulmaceae</i>	<i>rufofasciatus</i>	0	0	0	0	0	0	0	0
		<i>brevis</i>	1	0	1	2	24	4	13	41
	<i>Chamaeleontina</i>	<i>densifolia</i>	0	0	1	1	1	4	1	6
		<i>emetica</i>	92	58	21	171	13	45	58	216
	<i>Fraxillia</i>	<i>laurocerasus</i>	16	10	0	26	7	12	5	24
		<i>paludosa</i>	6	9	3	18	9	17	26	52
	<i>Rubus</i>	<i>rubescens</i>	4	7	6	17	4	6	3	13
		<i>sororia</i>	2	2	0	4	0	1	0	1
Thelephoraceae	<i>Thelephora</i>	<i>subglabripes</i>	2	0	0	2	0	0	0	2
		<i>variata</i>	59	35	51	145	11	20	6	39
	<i>Cantharellus</i>	<i>terrestris</i>	4	1	1	6	6	3	17	26
		<i>unifolius</i>	0	0	0	0	0	0	0	0
	<i>Gillocybe</i>	<i>gibbata</i>	53	128	100	281	2	10	2	12
		<i>laccaria</i>	23	26	53	102	12	28	45	85
	<i>Tricholoma</i>	<i>triviale</i>	0	15	0	15	0	0	0	15
		<i>resplendens</i>	3	0	0	3	0	2	14	16
	<i>Tricholoma</i>	<i>triviale</i>	0	0	0	0	0	0	0	0
		<i>resplendens</i>	0	0	0	0	0	0	0	0

a. Presence detected by association with *Cordiceps* spp. fruiting bodies.

Tables 29 and 30 present the current status of the reference sporocarp and culture collections. Most of the presumed mycorrhizal fungi fruiting at or near the sites are included in the sporocarp collection. Both collections have been improved substantially this past year.

Table 29. Species of presumably mycorrhizal fungi currently represented in the freeze-dried specimen collection.

Family	Genus	Species	Year Acquired
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u> Atk.	1983
		<u>brunnescens</u> Atk.	1983
Boletaceae	<u>Amanitopsis</u>	<u>citrina</u> (Schaeff ex) S.F. Gray	1983
		<u>muscaria</u> (Fr.) S.F. Gray	1984
	<u>Limacella</u>	<u>vaginata</u> (Bull. ex Fr.) Vitt.	1983
		<u>tilinita</u> (Fr.) Earle	1984
	<u>Boletellus</u>	<u>chrysenteroides</u> (Snell) Sing.	1984
		<u>russellii</u> (Frost) Gilbert	1984
	<u>Boletus</u>	<u>piperatus</u> Bull. ex Fr.	1983
		<u>subglabripes</u> Pk.	1984
	<u>Leccinum</u>	<u>undetermined "A"</u>	1983
		<u>insigne</u> Smith, Thiers & Watling	1983
Cantharellaceae	<u>Suillus</u>	<u>scabrum</u> (Bull. ex Fr.) S.F. Gray	1983
		<u>granulatus</u> (L. ex Fr.) O. Kuntze	1983
Clavicipitaceae	<u>Cantharellus</u>	<u>chromapes</u> (Frost) Sm. & Th.	1984
		<u>tutescens</u> Fr.	1983
Cortinariaceae	<u>Cordyceps</u>	<u>capitata</u> (Fr.) Link	1984
		<u>ophioglossoides</u> (Fr.) Link	1984
	<u>Cortinarius</u>	<u>armillatus</u> (Fr. ex Fr.) Fr.	1983
		<u>callisteus</u> (Fr. ex Fr.) Fr. ^a	1983
	<u>Hebeloma</u>	<u>caninus</u> (Fr.) Fr. ^a	1983
		<u>cinnamomeus</u> Fr. ^a	1983
	<u>Rozites</u>	<u>flavifolius</u> Peck	1983
		<u>olivaceus</u> Peck	1983
	<u>Elaphomyces</u>	<u>pholideus</u> (Fr.) Fr.	1984
		<u>sanguineus</u> (Fr.) Fr.	1983
Elaphomycetaceae	<u>Gomphidiaceae</u>	<u>semisanguineus</u> (Fr.) Gill	1983
		<u>sphaerosporus</u> Pk.	1984
	<u>Hydnaceae</u>	<u>trivialis</u>	1983
		<u>crustuliniforme</u> (Bull. ex St. Am.) Quel.	1983
	<u>Hydrophoraceae</u>	<u>mesophaeum</u> (Pers.) Quel.	1983
		<u>caperata</u> (Pers. ex Fr.) Karst.	1983
	<u>Paxillaceae</u>	<u>undetermined "A"</u>	1983
		<u>rutilus</u> (Fr.) Miller	1984
	<u>Polyporaceae</u>	<u>zonatum</u> (Fr.) Karsten	1983
		<u>imbricatum</u> Fr.	1983
Phodophylliaceae	<u>Phodophyllus</u>	<u>borealis</u> (Peck) Murr.	1983
		<u>aurantiaca</u> (Mulfen ex Fr.) R. Maire	1983
	<u>Rhodophyllus</u>	<u>perennis</u> Fr. (Muff.)	1984
		<u>lividus</u> (Bull. ex Merat) Quel.	1983

Table 29 Con't. Species of presumably mycorrhizal fungi currently represented in the freeze-dried specimen collection.

Family	Genus	Species	Year Acquired
Russulaceae	<u>Lactarius</u>	<u>griseus</u> Pk.	19884
		<u>affinis</u> Pk.	1984
		<u>argillaceifolius</u> Hesler & Smith	1983
		<u>funosus</u> Peck	1983
		<u>resinus</u> (Fr.) Fr.	1984
		<u>rufus</u> (Fr.) Fr.	1984
		<u>subvellerus</u> Peck var. <u>subdistans</u> Hesler & Smith	1983
		<u>torminosus</u> (Fr.) S.F. Gray	1984
		<u>uvidus</u> (Fr.) Fr.	1984
		<u>vinaceorufescens</u> Smith	1983
		<u>acruginea</u> Lindblad apud Fr.	1983
		<u>albida</u> Peck	1983
		<u>albidula</u> Pk.	1984
	<u>Russula</u>	<u>albonigra</u> (Krumh) Fr.	1983
		<u>amygdaloides</u> Kauffm. ^a	1983
		<u>brevipes</u> Peck	1983
		<u>chamaeleontina</u> Fr. sensu Schaffera	1984
		<u>densifolia</u> (Secr.) Gillet	1984
		<u>emetica</u> (Schaff. ex Fr.) Pers. ex Fr.	1983
		<u>fragilis</u> (Pers. ex Fr.) Fr. ^a	1983
		<u>laurocerasi</u> Melzer	1983
		<u>paludosa</u> Britz.	1983
		<u>rubescens</u> Beardslee	1983
		<u>sororia</u> (Fr.) Romell	1984
		<u>squalida</u> Peck	1983
		<u>subdepallens</u> Pk.	1984
Thelephoraceae Tricholomataceae	<u>Thelephora</u> <u>Clitocybe</u>	<u>tenuiceps</u> Kauffm. ^a	1983
		<u>variata</u> Bann. apud Peck	1983
		<u>xerampelina</u> (Schaff. ex Secr.) Fr.	1983
		<u>terrestris</u> Fr.	1984
		<u>gibba</u> (Pers. ex Fr.) Kummer	1983
		<u>hydrogramma</u> (Bull. ex Fr.) Kummer	1984
		<u>ordora</u> (Bull. ex Fr.) Kummer	1983
		<u>taccata</u> (Scop. ex Fr.) Berk. & Br.	1983
		<u>undetermined "A"</u>	1983
		<u>flavovirens</u> (Pers. ex Fr.) Lundell apud Lund. & Nannf.	1983
		<u>resplendens</u> (Fr.) Quel.	1984
		<u>Laccaria</u> <u>Leucopaxillus</u> <u>Tricholoma</u>	

a. Specific epithet uncertain.

Table 30. Results of isolations attempted from fruiting bodies of presumed mycorrhizal fungi occurring on study sites.

Family	Genus	Species	Year Attempted	Result
Amanitaceae	<u>Amanita</u>	<u>bisporigera</u> Atk.	1984	+
		<u>brunnescens</u> Atk.	1983, 1984	+
		<u>citrina</u> (Schaeff. ex) S.F. Gray	1983	+
		<u>muscaria</u> (L. ex Fr.) Pers. ex Hooker	1983	+ b
			1984	+
Boletaceae	<u>Boletellus</u>	<u>vaginata</u> (Bull. ex Fr.) Vitt.	1983	-
		<u>chrysentheroides</u> (Snell) Singer	1984	+
		<u>russellii</u> (Frost) Gilbert	1984	+
		<u>piperatus</u> Bull. ex Fr.	1983	+ b
			1984	+
	<u>Boletus</u>		1984	+
		<u>ornatipes</u> Peck	1984	+ c
		<u>subglabripes</u> Peck	1983	+
		<u>insigne</u> Smith, Thiers, & Watling	1983	+ b
		<u>scabrum</u> (Bull. ex Fr.) S.F. Gray	1983	+ c
Cantharellaceae Cortinariaceae	<u>Suillus</u>	<u>granulatus</u> (L. ex Fr.) O. Kuntze	1983	+
		<u>luteus</u> (Fr.) S.F. Gray	1984	+ c
	<u>Tylopilus</u>	<u>chromapes</u> (Frost) Smith & Thiers	1984	+
		<u>tutescens</u> Fr.	1984	+
	<u>Cantharellus</u>	<u>flavifolius</u> Peck		-
		undetermined "B"		-
	<u>Cortinarius</u>	<u>crustuliniforme</u> (Bull. ex St. Am.) Quel	1983, 1984	+
		<u>mesophaeum</u> (Pers.) Quel.	1983	+
	<u>Hebeloma</u>	undetermined "A"	1983	-
		<u>caperata</u> (Pers. ex Fr.) Karst.	1983	-
Paxillaceae	<u>Rozites</u> <u>Hygrophoropsis</u>	<u>aurantiaca</u> (Wulfen ex. Fr.) R. Maire	1983	+
			1984	+ b
Rhodophyllaceae Russulaceae	<u>Paxillus</u>	<u>involutus</u> (Fr.) Fr.	1984	-
		<u>griseus</u> (Peck)	1984	+
	<u>Rhodophyllus</u>	<u>argillaceifolius</u> Hesler & Smith	1983, 1984	+
		<u>piperatus</u> (L. ex Fr.) S.F. Gray	1983	-
	<u>Lactarius</u>	<u>resimus</u> (Fr.) Fr.	1984	+
		<u>rufus</u> (Fr.) Fr.	1984	+ b
		<u>subvellerens</u> Peck var. <u>subdistans</u>		
		Hesler & Smith	1983	+
	<u>Russula</u>	<u>vinaceorufescens</u> Smith	1983	+
			1984	+ b
		<u>acruginea</u> Lindblad apud Fr.	1983	-
		<u>variata</u> Bann. apud Peck	1983	-

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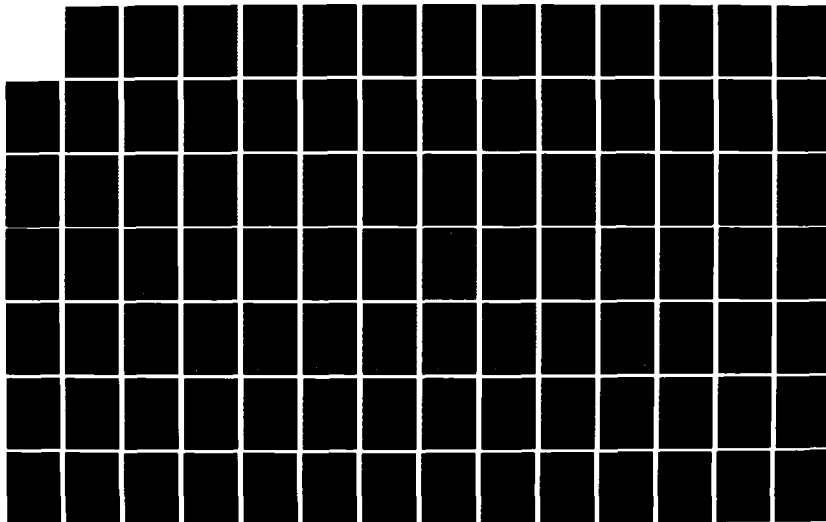
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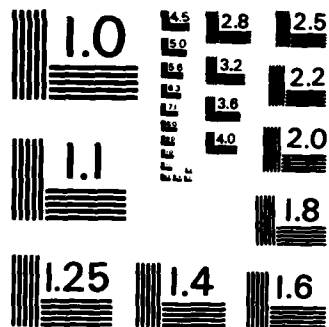
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Table 30 Con't. Results of isolations attempted from fruiting bodies of presumed mycorrhizal fungi occurring on study sites.

Family	Genus	Species	Year Attempted	Result
Sclerodermataceae	<u>Scleroderma</u>	<u>aurantium</u> (Vaill.) Pers.	1984	+b
		<u>bovista</u> Fr.	1984	+b
Thelephoraceae	<u>Thelephora</u>	<u>terrestris</u> Fr.	1984	+b
Tricholomataceae	<u>Clitocybe</u>	<u>gibba</u> (Pers. ex Fr.) Kummer	1983, 1984	+
		<u>hydrogramma</u> (Bull. ex Fr.) Kumer	1984	+
		<u>odora</u> (Bull. ex Fr.) Kummer	1983	+
	<u>Laccaria</u>	<u>taccata</u> (Scop. ex Fr.) Berk. & br.	1983	+b
				1984
		<u>Tricholoma</u>	<u>flavovirens</u> (Pers. ex Fr.) Lundell apud Lund. & Nannf.	1983
		<u>resplendens</u> (Fr.) Quel.	1984	+

a. Successfully isolated into pure culture (+); unsuccessful (-).

b. Occurring on study site, but isolated from other collections.

c. Not yet found on study site, but isolated for reference.

Element 7. Mycorrhiza Characterization and Root Growth

For reasons discussed in the 1983 Annual Report under Element 7, bare-root seedling red pine plantations have become the focus of attention for this work element. Mycorrhizal root tips are being quantified and characterized for red pine seedlings used for plant moisture stress and foliar nutrient analyses (Element 3). All outplanted seedlings were originally obtained from a single nursery bed at the Toumey National Forest Nursery in Watersmeet, Michigan. The seed source for the seedlings was Dickinson County, Michigan. Mycorrhizae occurring on red pine seedlings in the source nursery beds were studied in 1983, and those results were presented in the 1983 Annual Report under Element 7. The plantation sites offer ideal conditions for establishment of red pine, including abundant natural inoculum for mycorrhiza development. This is especially true because 1) no chemical site preparation was administered prior to planting, 2) planting was accomplished immediately subsequent to site clearing, and 3) ectomycorrhizal tree species are well-represented in both the pre-existing and surrounding stands.

The experimental design for data analysis remains basically as originally documented. Populations of mycorrhizae developing at each plantation site are being compared with each other at monthly intervals. The basic population units are individual mycorrhizal root tips, and the field sampling units are individual seedlings. Only viable mycorrhizae are counted. Individual mycorrhizae are categorized into morphological types which are presumed to represent different fungal associations with red pine. Changes over time, within and between sites, in the partial

frequencies of occurrence for different mycorrhizal rootlet morphology types will be quantified, as will changes in total numbers of mycorrhizae. Data for analysis will be expressed both as average mycorrhizal root tip counts per seedling and as numbers of mycorrhizae per gram (oven dry weight) of seedling root mass. Trends detected in mycorrhizal population development will be evaluated in light of the ambient monitoring data collected for each plantation site. Developmental patterns for mycorrhiza populations should serve well to detect possible ELF effects, because of the large sample sizes and the uniformity of planting stock used. Due to the delicate physiological balance between hosts and their mycorrhizal fungus associates, patterns of mycorrhiza community development may be expected to respond to possible ELF effects on either the host or its beneficial parasites.

The numbers and types of fungi responsible for producing the mycorrhiza types observed are also being investigated, since there is a direct relationship between the fungi available and the types of mycorrhizae produced. The frequency of occurrence of culturable mycorrhizal fungi is being estimated by isolating these organisms from active mycorrhizae using standard techniques.

Progress

As noted in Element 3, bare-root 3-0 red pine seedlings from the J.W. Toumey Forest Service Nursery in Watersmeet, Michigan, were planted on 1 m centers in clearcuts at all three study sites between 18-22 June, 1984. Fifty seedlings were randomly selected from the nursery storage bags at the time each site was planted to evaluate mycorrhizal condition of the planting stock. In the laboratory, roots were soaked in tap water for several hours and extraneous material was washed free in running water.

Viable mycorrhizae were counted and characterized with the aid of dissecting microscopes. Distinctive morphological types were separated and used for fungal isolations. Modified Melin-Norkrans (MMN) agar medium was used for isolations instead of Hagem's agar (HA) medium because MMN contains thiamine as well as all of the constituents of HA. More mycorrhizal fungi may be able to develop on MMN than on HA.

Forty-five randomly selected seedlings from each plantation site were excavated and processed as described above at monthly intervals after planting. Resulting mycorrhiza counts are presented in Table 31. These data show that nursery seedlings planted at all three sites initially carried large numbers of one type of mycorrhiza, and that these numbers decreased over the following two months. This decrease is attributed to planting shock as mycorrhiza numbers rebounded during September and October.

Only mycorrhizae of morphology type 3 were found on seedlings sampled at planting and in late July. Type 3 mycorrhizae also dominated 3-0 seedlings prior to lifting in the Toumey Nursery (Table 13, p. 57, 1983 Annual Report). Types 1 and 4 have since been determined to represent developmental stages of Type 3, based on microscopical and macroscopic evidence. Type 2 mycorrhizae, also found in the nursery, were not found at the plantation sites until late August. In addition, two new morphological types, Type 5 and Type 6, were found for the first time in late August. While Type 5 seems to be increasing in abundance on all three plantations, Type 6 is found sporadically and has yet to be observed on seedlings from the antenna ground site. The sporadic observation of Type 6 is attributed to its scattered distribution; the fact that it dominates those few

Table 31. Mean mycorrhiza counts by morphology type per gram (o.d.w.) of root mass for red pine seedlings sampled from each plantation site, beginning with plantation establishment in June, 1984.

Sampling Date	Study Site											
	Ground				Antenna				Control			
	Mycorrhiza Type				Mycorrhiza Type				Mycorrhiza Type			
	2	3	5	6 ^a	2	3	5	6	2	3	5	6
22 June	0	426 ^b	0	0	0	295	0	0	0	350	0	0
24 July	0	190	0	0	0	218	0	0	0	242	0	0
21 August	68	87	19	0	65	92	35	426	82	38	6	0
23 September	112	64 ^b	12	0	123 ^b	143	20	0	56	135	28	0
29 October	58	179	23	0	78	233	30	0	70	181	16	494

^a Type 6 mycorrhizae were not included in tests of significance because this type only occurred on a total of 3 seedlings on 2 of the sampling dates.

^b Treatment means which differ significantly from the Control means ($\alpha=0.05$).

seedlings with which it becomes associated explains the relatively high numbers of Type 6 mycorrhizae reported when it is collected. Types 5 and 6 mycorrhizae will likely become more abundant and more uniformly distributed at all three plantation sites as the seedlings become established in 1985.

Analysis of variance shows that the only significant difference ($\alpha = .05$) in total number of mycorrhizal root tips per seedling between the three plantations occurred at the time of planting. The seedlings sampled from the control site had fewer mycorrhizae than those planted at the two test sites. The control site was the last site to be planted and the delay was initially thought to have caused a loss of viable mycorrhizae. This initial difference between sites is not reflected in later counts. The utility of expressing mycorrhizae counts on the basis of root dry weight is evident from Table 32, which shows that the difference between sites in initial mycorrhiza counts per seedling is probably due to the slightly smaller mean size of seedlings planted at the control site, rather than to loss of viable mycorrhizae due to planting delay. Partial frequencies of occurrence for each mycorrhiza morphology type on each sampling date at each plantation will be characterized in greater detail, both on a seedling basis and on a seedling root weight basis. This will facilitate evaluation of type distribution and population dynamics within and between plantation sites.

Because the same seedlings are used for both mycorrhiza counts and plant moisture stress (PMS) measurements, the relationship between seedling moisture stress and mycorrhiza counts is being evaluated. Mycorrhiza counts for seedlings in the same condition class (See Element 3 for descriptions of condition classes) based on moisture stress should be more comparable and

Table 32. Mean numbers of mycorrhizal root tips per gram (o.d.w.) of root mass for red pine seedlings sampled from each plantation site, beginning with plantation establishment in June, 1984.

Sampling Date	Study Site		
	Ground	Antenna	Control
22 June	426 ^a	295	350
24 July	190	219	242
21 August	103	127	92
23 September	104	145	128
29 October	151	216	164

^a Treatment mean differs significantly from Control mean ($\alpha = 0.05$).

Table 33 Mean non-mycorrhizal root tip counts per gram (o.d.w.) of root mass for red pine seedlings sampled from each plantation site, beginning 2 months after planting.

Sampling Date	Study Site		
	Ground	Antenna	Control
21 August	68	46	64
23 September	24	21	20
29 October	9 ^a	18	18

^a Treatment mean differs significantly from Control mean ($\alpha = 0.05$).

provide a more rigorous test of differences between plantations or sampling dates. During 1984, however, analysis of variance did not show significant differences ($\alpha = .05$) between mycorrhiza counts for seedlings in condition class 1 from the three plantations. Additional analyses are being conducted and will be included in the final version of this report.

Non-mycorrhizal root tips were not found on the seedlings at the time of planting and few were observed in late July. Counts of non-mycorrhizal root tips were initiated in late August, at which time large numbers were encountered; these counts are summarized in Table 33. Non-mycorrhizal root tip numbers decreased greatly during late summer and autumn of 1984. Development of non-mycorrhizal root tips is assumed to reflect inadequacy of immediately available active mycorrhizal fungus inoculum as the planted seedlings began to grow at the plantation sites. The decrease in numbers of non-mycorrhizal root tips later in the summer reflect the increased inoculum potential available for mycorrhiza development. Counts of non-mycorrhizal roots will continue in 1985.

Twenty-four new isolates of presumed mycorrhizal fungi were obtained from the plantation red pine seedlings sampled in 1984. All except three of the 1983 Toumey Nursery isolates re-occurred on the plantation seedlings. The entire collection of isolates will be further characterized in the laboratory. Consideration of fruiting records at the study sites in conjunction with records of isolation attempts from sporocarps (Element 6) will indicate which mycorrhizal fungus species may or may not be expected to develop in pure culture from mycorrhizal root tips. Comparison of cultures from mycorrhizal root tips with those from identified fruiting bodies will permit identification of a portion of the root isolates. The frequency with

which specific presumed mycorrhizal fungi are isolated from the various mycorrhiza morphology types will help to further characterize the mycorrhizal relationships represented by each type.

Element 8. Litter Production

Litter fall and decomposition is important in the transfer of nutrients and energy within a vegetative community. The sensitivity of foliage production to both internal and external conditions make it a good indicator of possible ELF field effects on trees. Since litter samples can be gathered at frequent intervals, they not only provide an estimate of changes in canopy production, but also give an insight on tree phenological events such as leave fall, bud burst, seed, dissemination, and time of flowering. Additionally, leaf samples taken during the growing season for nutrient analysis and weight determination would monitor nutrient accumulation and subsequent translocation from the foliage to the branches prior to leaf fall. This physiological process is also sensitive to environmental stress and would be a potential indicator of ELF field effects.

Progress

Five 1x1 meter litter traps are being used to monitor tree litter production on each permanent measuring plot at the antenna and the control sites. The fifteen traps previously established on the original control site were moved to the new control in May 1984. Litter was collected at intervals during the summer and weekly after the onset of leaf fall in early September.

In last years sampling foliage was taken from big tooth aspen, paper birch, red maple, and northern red oak. However, an examination of the nutrient concentrations in these leaves showed that, red oak foliage had the least nutrient variation of the four species tested. Consequently, northern red oak was selected for an intensive study of crown nutrient concentrations and translocation. Foliage samples were collected from more

northern red oak trees at both the antenna and control site in August, September and October. An analysis of stem diameter data indicated that sampling trees of 15 cm, 21 cm and 32 cm would adequately represent the distribution of red oak on each site. Three trees of each diameter were located off of the permanent measurement plots at each site to minimize disturbance. Leaf samples were obtained from near the top of the crown using a 12 gauge shotgun with a full choke.

All litter and foliage samples were dried at 60⁰C in a forced draft oven. The litter was separated into the following categories: (1) leaves, (2) wood, (3) miscellaneous and weighed. A representative subsample of ten leaves was taken from each foliage collection and weighed. All samples were ground to pass a 40 mesh sieve and are being analyzed for N by Kjeldahl digestion, P by the vanadomolybdate method, and Ca, Mg and K by atomic absorption.

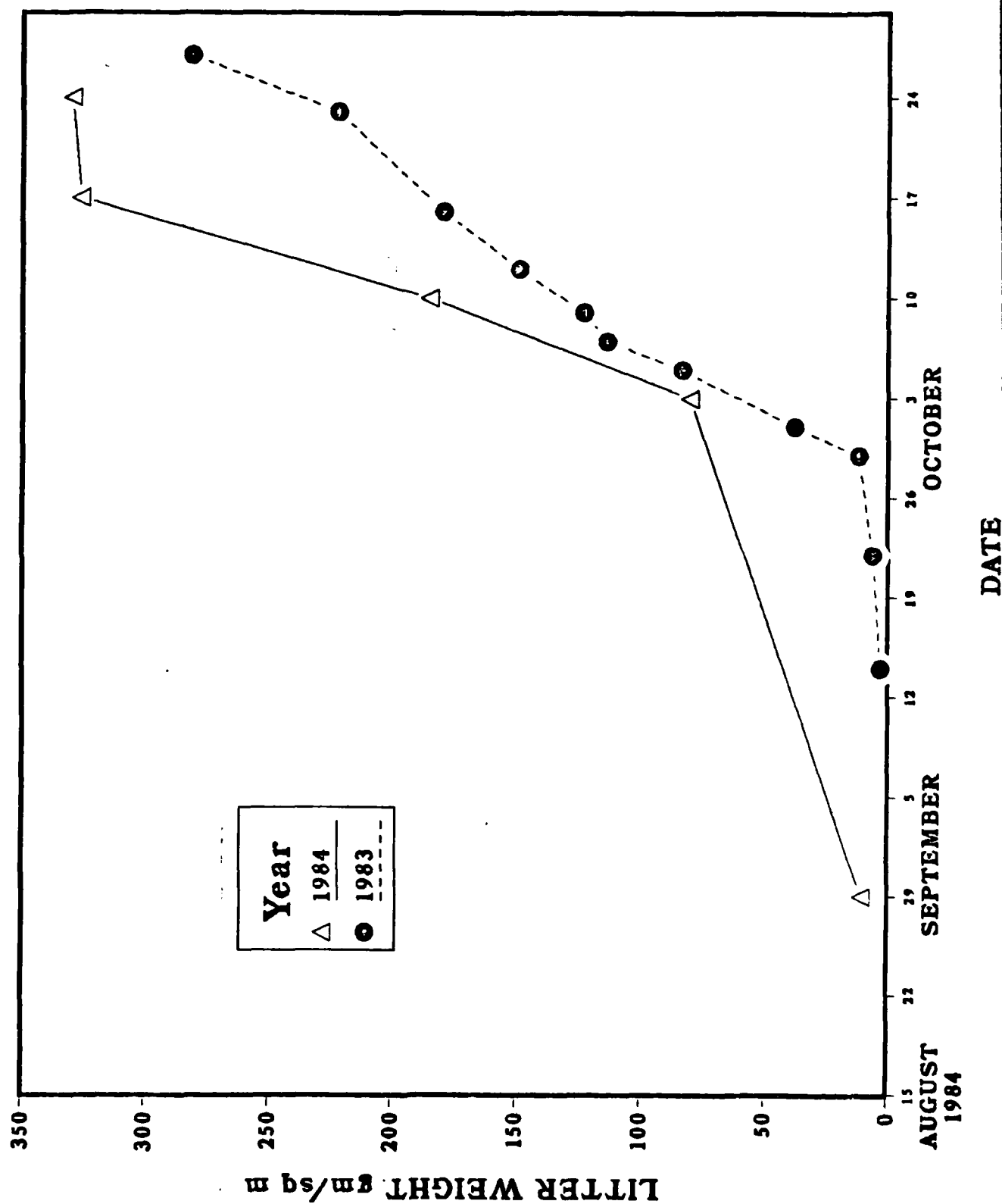
Litter fall on the antenna site this year was much earlier when compared to 1983 (Figure 11). Nearly all of the leaves had fallen by the 10th of October at both locations (Figure 12). A significant difference in total leaf litter was found between the antenna and control sites (Table 34). Woody and miscellaneous tree components were a minor part of the total litter fall and only small differences were evident between the sites. The error estimate of the mean for total leaf litter fall was 36.6 g/m² for the antenna site and 29.6 g/m² for the control site, or an error percent of 11.9 and 8.2 of the mean at $\alpha = 0.05$, respectively. This error value is well within acceptable variability standards for litter production studies and indicates that no additional litter traps are needed for monitoring possible ELF effects. While it is desirable not to have significant litter differences between the two study sites, covariate analysis can be used to separate possible ELF effects from the inherent site variation shown here.

Periodic litter fall amounts varied significantly ($P < 0.05$) between the antenna site and the control site at all collection times in the fall (Figure 13). These differences in weekly leaf fall were related to variable tree species composition at each site. The antenna site has a much higher proportion of dominant red maple and big-toothed aspen than the control. In contrast northern red oak predominates on the control site. Oak leaves remained on the trees longer than the maple or aspen and accounted for much of the litter fall variations between locations. This species variability in leaf retention is discussed more fully in Element Tree Phenology.

The average leaf weight of northern red oak did not show significant differences ($p < .05$) between the two sites at any of the three sampling dates (Table 35). No relationship was found between sample tree size and average leaf weight. Nutrient analyses of the foliage and litter samples are being conducted and will be used to calculate total nutrient losses to the trees from litterfall.

CUMULATIVE LITTER PRODUCTION ANTENNA SITE

Figure 11.



LEAF LITTER PRODUCTION 1984

Figure 13.

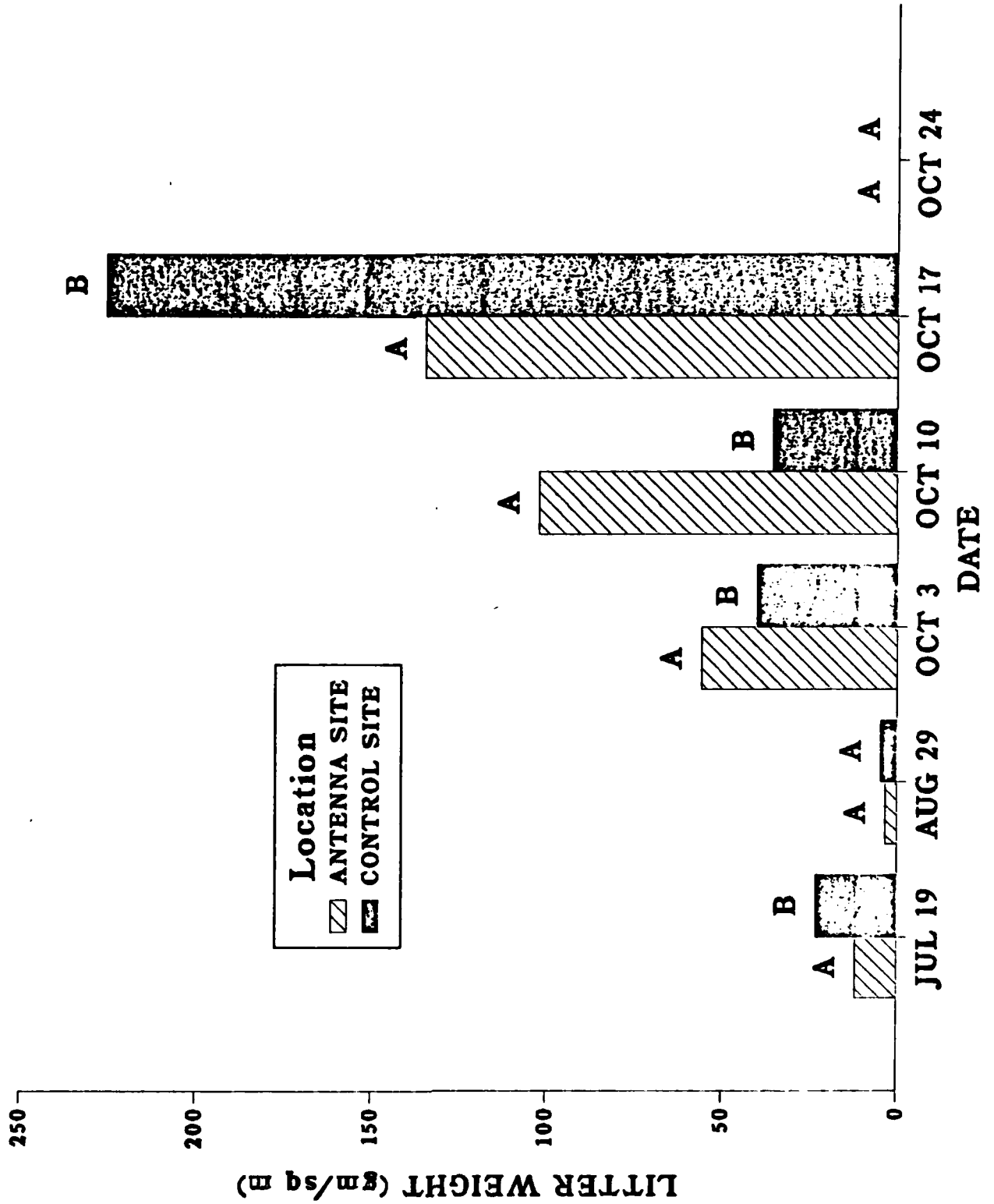


Table 34. Total Litter Fall at the Antenna and control Sites.

Site	Total Litter Fall		
	Leaves	Wood (gm/m ²)	Misc.
Antenna 1983*	264.1 ^A	7.9 ^L	3.5 ^X
1984 ⁺	307.2 ^A	15.0 ^L	19.0 ^Y
Control 1984 ⁺	341.8 ^B	10.1 ^L	27.3 ^Y

* Litter collection period from August to November.

+ Litter collection period from June to November.

Table 35. Average Weight of Northern Red Oak Leaves at Aboveground Antenna and control Sites.

Site	Ave. Leaf Wgt. (gm)	Standard Deviation	Error %
Antenna	0.52	1.49	11.4
Control	0.55	1.22	8.8

ELEMENT 9 DATA MANAGEMENT

The study design of the Trees and Herbaceous Plants and Litter Decomposition Tasks requires the integration of ambient monitoring data with biological data in statistical analysis in order to separate environmental factors from possible influences due to ELF electromagnetic fields. To facilitate this process SIR DBMS (Scientific Information Retrieval Database Management System) was purchased. The database will allow the handling and manipulation of large quantities of data as well as defining specific formats for each data set. This will insure uniformity and compatibility in integrating data sets within statistical analysis. In addition SIR provides a convenient system for long term storage of data sets.

Meetings were held with each principle investigator to review the type, amount and frequency of data being collected. As a result, the initial database structure developed in 1983 has been revised in 1984 and is shown in Appendix K. These revisions include the addition of two data sets: 1) red pine seedling measurements (See Element 3 - Tree Productivity); 2) red pine phenology (See Element 4 - Phenophase Description and Documentation). The tree phenology data set has been discontinued as a result of the elimination of several phenological events from the study (see Element 4 - Phenophase Description and Documentation). Cambial activity and leaf fall will be retained and data collected on these events will be stored in the dendrometer band and litter trap data sets.

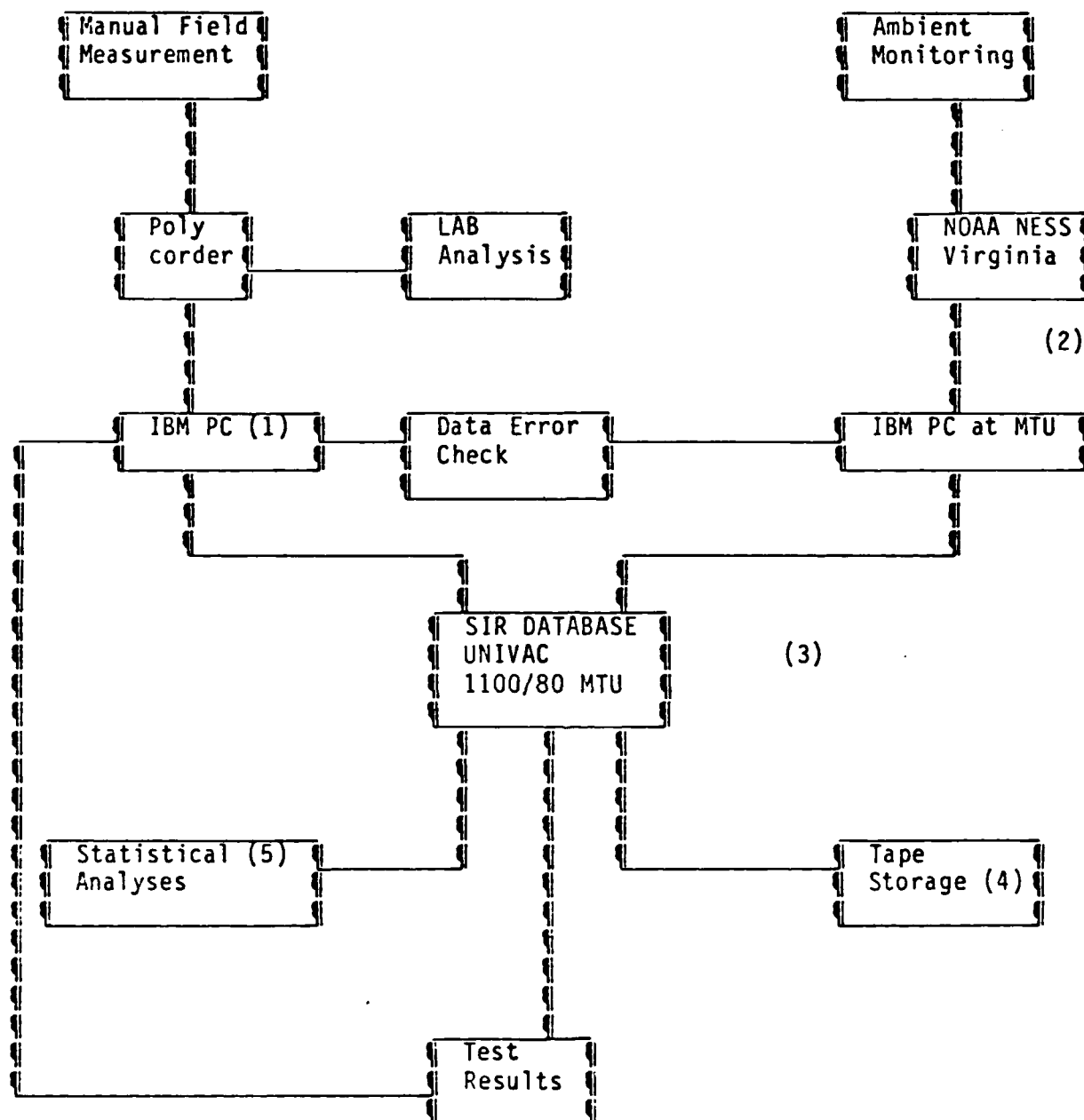
The data management plan for both the Trees and Herbaceous Plants and Litter Decomposition Tasks remains unchanged from 1983 and is shown in Figure 14. The field measurements are entered on a Polycorder (an electronic data loggers device), or samples collected and tagged for further

analysis. Data entered on the Polycorder is then transferred to the on site IBM Personal Computer (1) for error checking. Laboratory analysis data is also entered and error checked on the IBM PC as it becomes available. Data is then transferred from the on site IBM PC to the UNIVAC 1100/80 SIR Database using a 1200 baud asynchronous dialup line.

The daily ambient monitoring information is transferred via satellite to Virginia (NESS) for initial storage (refer to Element 2). A 300 baud asynchronous dialup phone line is used to transfer data from NESS daily to an IBM PC at Michigan Technological University (2, Figure 5). The IBM PC will then transfer the data using a 1200 baud asynchronous dialup line to the UNIVAC 1100/80 SIR Database.

The UNIVAC 1100/80 SIR Database (3) is then used to store, retrieve and aid in the analysis of both the ambient monitoring and field information. Data will be intermittently written to tape (4) for backup. In addition, data not currently being analyzed will be unloaded from the database and placed on tape to reduce file storage costs.

Figure 14. DATA MANAGEMENT FLOWCHART



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APPENDIX A

Summary report of vandalism
at the Trees and Herbaceous
Plant Cover Control Site.



IIT Research Institute
10 West 35 Street, Chicago, Illinois 60616
312/567-4000

5 November 1984

TO: Naval Electronics Systems Command, PME 110-EB

FROM: IIT Research Institute

Prepared by:

Approved by:

Two handwritten signatures are present. The first signature is written over the "Prepared by:" line and the second is written over the "Approved by:" line. Both signatures are in black ink and appear to be cursive or semi-cursive.

SUBJECT: Vandalism to Ecological Monitoring Sites - Michigan Technological University

Over the last two years there have been several incidents of vandalism to study sites occupied by the Ecological Monitoring Program. In late summer, 1983 boundary and quadrat marking stakes were removed at a Michigan State University (MSU) site (Soil Arthropod and Earthworm Study). This summer equipment was removed and emplaced in a nearby swamp (MSU-Bee Study). Most recently, two study sites used by Michigan Technological University (MTU) have been extensively damaged. This memorandum documents the background, damage and current status of the MTU sites.

Background

Michigan Technological University is studying physiological, population and community aspects of upland flora (trees, herbs, fungi and soil bacteria). One control site (Paint Pond Road) is located in Iron County, Michigan and two test sites (Martell's Lake - Overhead; Martell's Lake - Buried) are located in Marquette County, Michigan (see Attachment 1). All sites are marked with signs indicating them as MTU environmental monitoring sites. On 16 October 1984 the control site was vandalized in the late morning and possibly early afternoon hours. Subsequently, between 24 and 29 October, the Martell's Lake - Overhead Test Site was damaged.

Damage

Vandalism at the Test Site consisted of removal of 15 stakes marking the boundaries of study plots within the site. The Control Site was more extensively damaged (see Attachment 2). Repair costs at the Control Site are estimated at \$29,000 (see Attachment 3). Repairs at the Test Site should be roughly equivalent to replacing boundary markers at the Control Site, i.e. \$700. As of 30 October 1984, the Martell's Lake - Buried Study Site had not been harmed.

The vandalism has not strongly affected collection of scientific data for 1984 as the field season was nearly complete. There will be some loss in the continuity of data between 1984 and 1985, particularly for the herbaceous phenology, and foliar analysis, study elements. In addition several project personnel are adversely affected as they are using the data for postgraduate thesis research.

Current Status

The Iron County Sheriff's Department has an ongoing investigation of the vandalism at the Control Site; their report is enclosed (Attachment 4). The Marquette County Sheriff's Department was contacted on 31 October concerning the damage at the Test Site. Cost estimates (Attachment 3) have been submitted to the MTU Research Office and will be made available to the Iron County Sheriff after administrative review. Photographs of the damage have been taken by MTU personnel and copies will be forwarded to the Project Office when available. Sites are currently being restored, however several study elements cannot be reestablished with the Spring of 1985.

Enclosures (4)

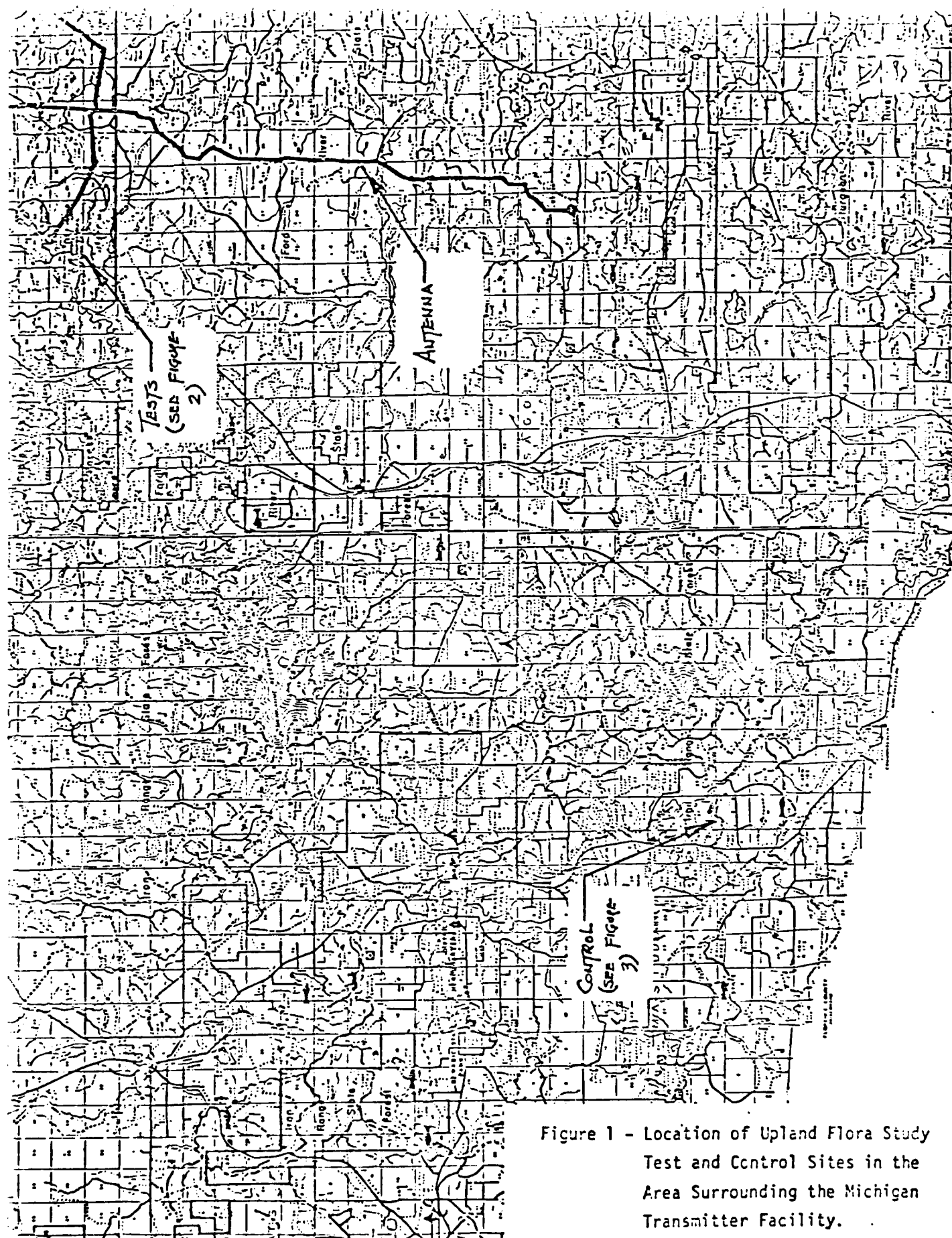
cc: PME 110-EC
Lt. C. Walker
J. Maurer, MDNR
{ G. Mroz
M.M. Abromavage
R. Carlson/file

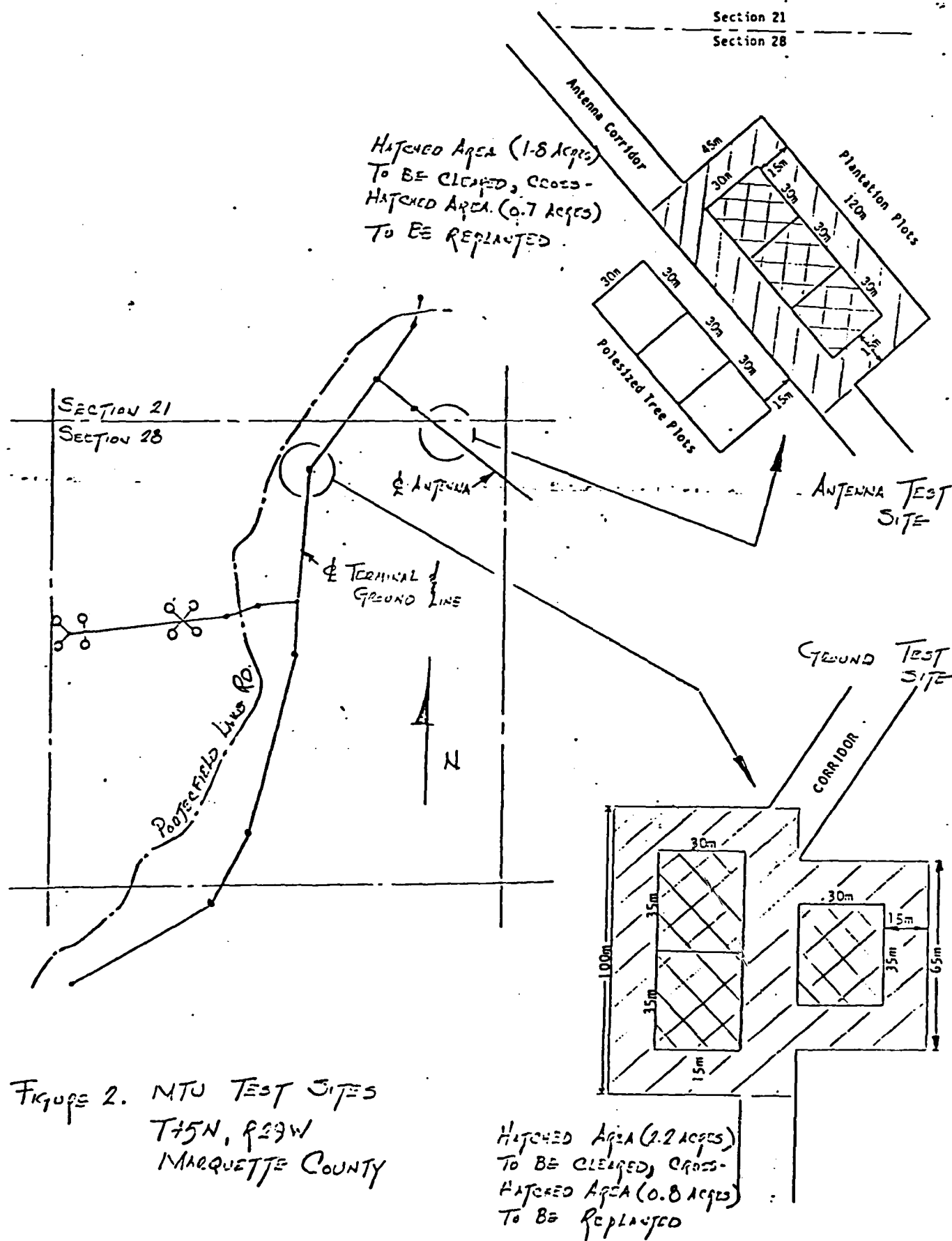
ATTACHMENT 1

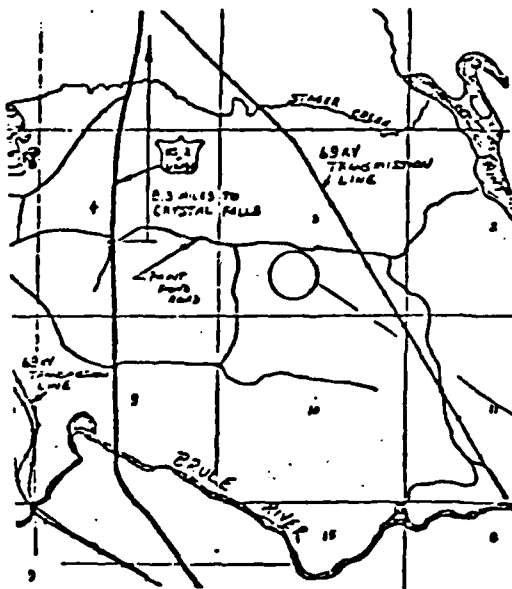
LOCATION OF UPLAND FLORA
STUDY SITES

IIT RESEARCH INSTITUTE

/41







HATCHED AREA (1.8 ACRES)
TO BE CLEARED, CROSS-
HATCHED AREA (0.7 ACRES)
TO BE REPLANTED

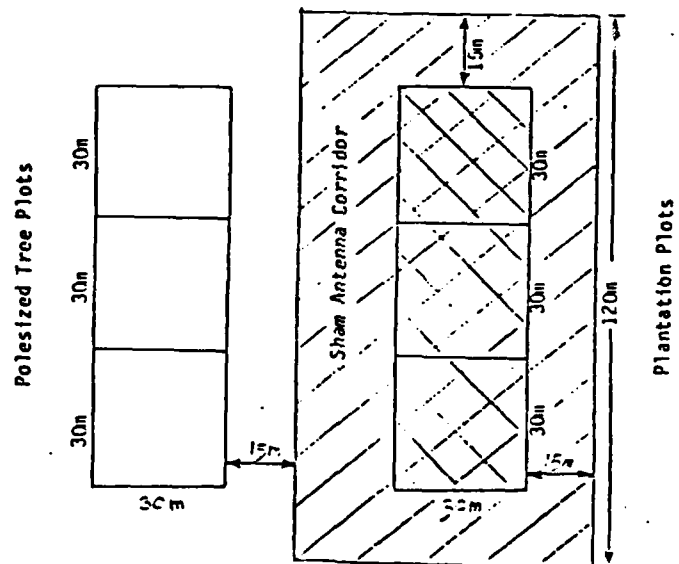


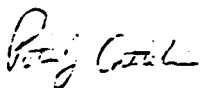
FIGURE 3. MTU CONTROL SITE
T41N, R32W
IRON COUNTY

CONTROL SITE

**LOCATION OF DAMAGE TO MTU
PAINT POND ROAD STUDY SITE**

Location of various damages to the Michigan Tech ELF research site located near the Paint Pond Road can be found on the attached map of the research area. Numbers circled on the map correspond to the damages listed below.

1. On plot 312, the solar panel on transmitter housing was unbolted and placed on the ground. Rain gauge was pulled out of the ground, moved approximately 20 feet, and layed under the transmitter housing. Cable on the relative humidity-air temperature sensor was disconnected.
2. Two cables from the ambient system junction box to the soil temperature and moisture probes on plot 313 are missing. Cable on air temperature sensor was disconnected.
3. Plot corner stakes on the red pine plantation were pulled and tossed around.
4. An unknown number of stakes marking red pine seedlings were pulled and tossed around.
5. 172 out of 274 dendrometer bands were destroyed.
6. 38 out of 40 permanent meter square plots used for herbaceous plant measurements were destroyed.
7. Plot corner stakes on the pole-sized tree plots were pulled and tossed around.
8. Two of 15 litter traps were moved.
9. 5 out of 9 tree identification tags were removed from trees used for foliage sampling.
10. All plot corner and transect stakes on the herbaceous plant reserve were pulled and tossed around. All flagging marking the transects was also removed.
11. Four soil temperature and moisture probes on plot 322 were pulled out of the ground.



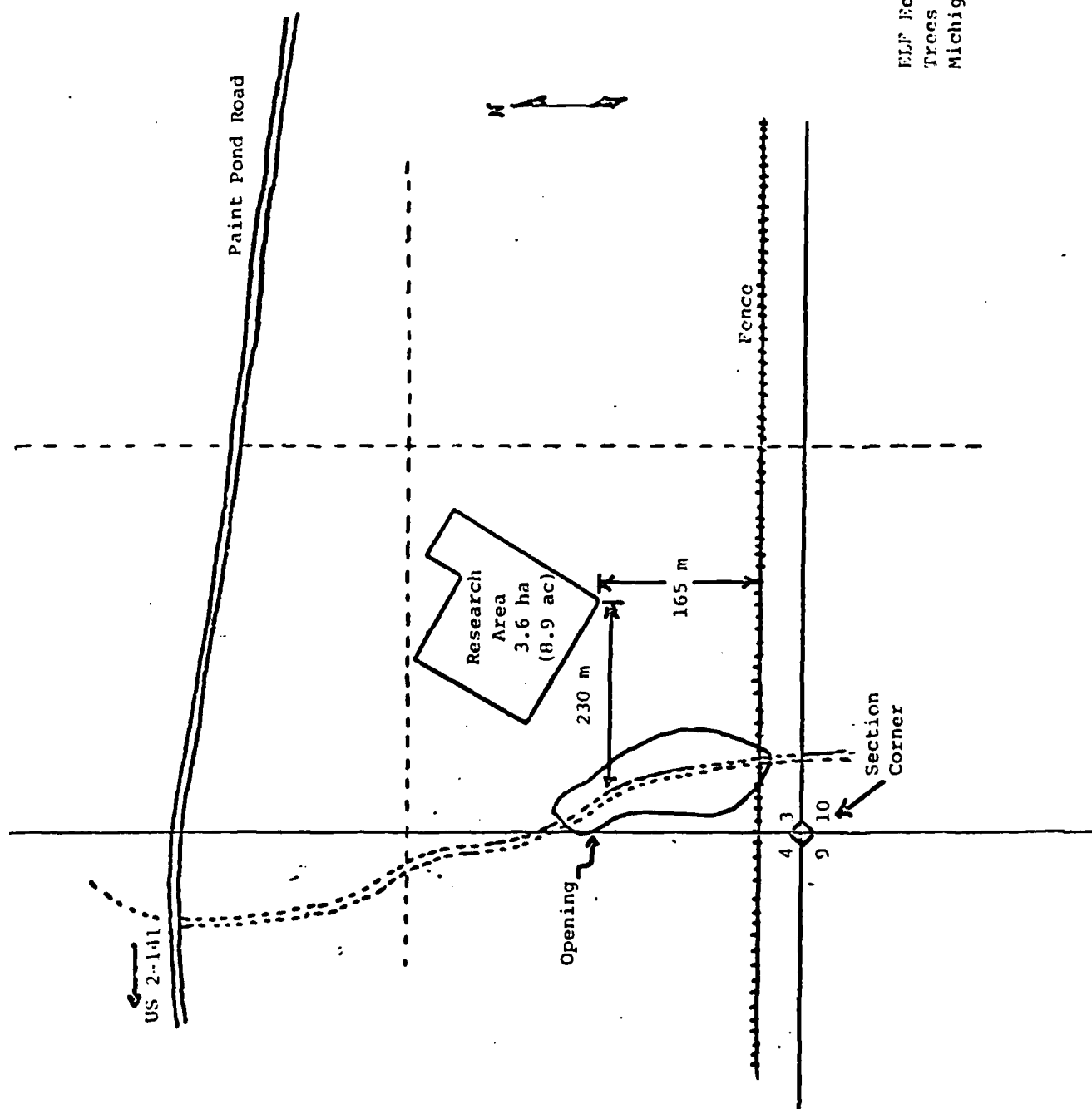
Peter J. Cattelino
Research Associate

October 22, 1984

ELF CONTROL PLOTS
(Paint Pond Road)

SW 1/4, SW 1/4. Sec. 3
T41N - R32W

ELF Ecological Monitoring Program
Trees and Herbaceous Plants Study
Michigan Technological University

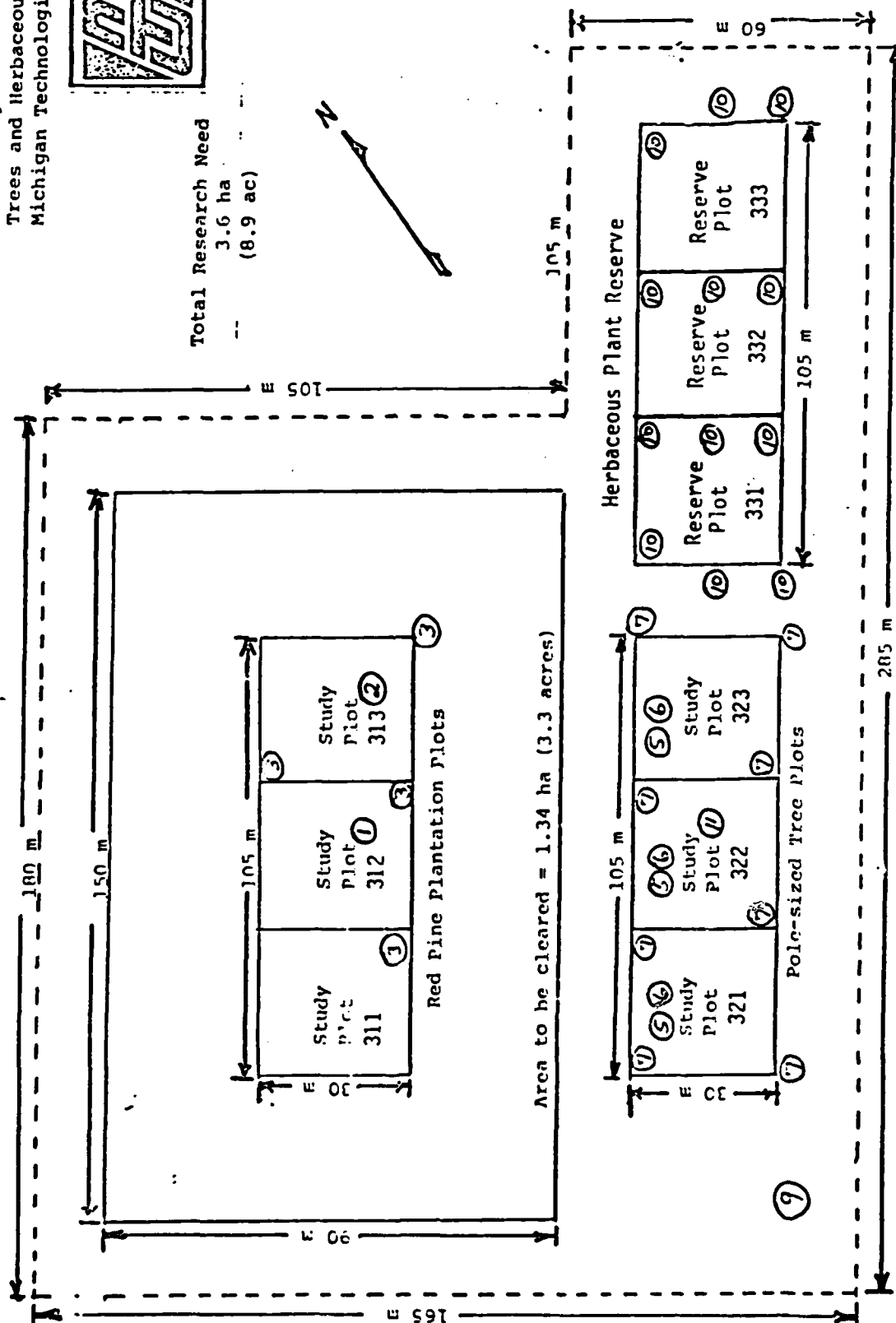


ELF Control Plots
(Paint Pond Road)
SW 1/4, SW 1/4, Sec. 3
T41N - R32W

**ELF Ecological Monitoring Program
Trees and Herbaceous Plants Study
Michigan Technological University**



	Total Research Need
--	3.6 ha
	(8.9 ac)



ESTIMATE OF VANDALISM DAMAGE

to ELF Environmental Monitoring Program
Study Sites for Trees and Herbaceous Plant Cover

HERBACEOUS PHENOLOGY

Herbaceous phenology studies were seriously impaired with the destruction of 38 of 40 plots. The locations of these are not known because the plants are no longer present on the sites. We will have to wait for emergence in the spring to reinstall these. However, by the time the plots can be reestablished, we will have missed the phenophases in emergence. In addition, using different plots for these studies next year continues to be a cause of concern. At this time we estimate the value of damage as the direct costs incurred this year. This cost is not reimbursable at this time.

a. Jan Schultz	
150 hrs. @ \$12.63	1,894
b. Thompson Hill	
128 hrs. @ \$4.59	587
c. Pete Cattelino	
60 hrs. @ \$8.85	531
d. Beth Reed	
30 hrs. @ \$8.99	270
Fringes 26% of c & d	208
3% of b	18
Travel 1600 miles @ .25/mile	<u>400</u>
	3,908
35% OH	<u>1,368</u>
	\$5,276

FOLIAR ANALYSES

Flags and labels were removed from hardwood trees used for foliage sampling. New trees will have to be selected as these were scattered around the plots. We will need to identify similar trees (based on crown position and diameter) and find how next year's data compares to the baseline on treatment and control plots. If we find the same temporal relation in nutrient values across the study plots, this past year's data will be useful. If not, it will be of diminished value. That value is estimated by the costs of sampling and analyses given below. This value is not reimbursable at this time.

Martin Jurgensen	
24 hrs. @ \$23.28	559
Glenn Mroz	
24 hrs. @ \$13.81	<u>331</u>
	890
Fringes @ 19.6%	<u>174</u>
	1,064
Analyses	<u>5,380</u>
	6,444
+35% Indirect Cost	<u>2,255</u>
	\$8,699

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Travel 1600 miles @ .25/mile	<u>400</u>
	3,908
35% OH	<u>1,368</u>
	\$5,276

DENDROMETER BANDS

Portions of productivity and phenology studies depend on individual tree diameter increment measurements. Vandalism involving the removal of 172 of 274 bands along with tree identification tags impairs each of these studies. Replacement of these and re-identification of the trees is imperative. This will entail exact measurement (to the closest .01 inch) of the trees, matching these diameters to the last dendrometer band reading and re-fitting the trees.

a. Glenn Mroz		
80 hrs. @ \$13.82		1,106
b. Peter Cattelino		
160 hrs @ \$8.85		1,416
c. Beth Reed		
80 hrs @ \$8.99		719
d. Thompson Hill		
160 hrs @ \$4.60		736
e. Gary Lenz		
160 hrs @ \$7.40		<u>1,184</u>
		5,161

Fringes

26% of b,c,e	3319 =	863
19.6% of a	1105 =	217
3% of d	736 =	<u>22</u>
		1,102

Per Diem	753
Mileage 700 miles \$.35 a mile	245

Materials

Stainless Steel	250
Springs @ .60/each	103
Tags	<u>20</u>
	7,634
+ 35% OH	<u>2,672</u>

\$10,306

PLOT BOUNDARY MARKERS

Most plot boundary stakes were removed from the study areas. In addition, flagging marking frequency and coverage transects in the herbaceous reserve plots were removed. These can be replaced and costs are listed below:

Peter Cattelino	
24 hrs. @ \$8.85	212
Gary Lenz	
24 hrs. @ \$7.40	<u>178</u>
	390
+ 26% Fringes	<u>101</u>
	491
Plot Travel	
150 miles @ .35	<u>52</u>
	543
@ 35% OH	<u>190</u>
	\$733

AMBIENT MONITORING

Damage to ambient systems will require reconstruction of several cables, reinstallation of some equipment and recalibration of sensors. In addition, several main cables received damage of unknown severity. These will have to be tested. The estimated cost of this work is shown below.

<u>1 Week</u>	
Dave Wilson 40 @ \$10.86	435
Mark Anderson 40 @ \$11.68	467
Carl Trettin 24 @ \$14.23	342
Dennis Olsen 32 @ \$10.13	<u>324</u>
	1,568
Fringe 26%	408
Cables and Connector Replacement	<u>480</u>
	2,456
Travel 300 miles @ .35 miles	105
Per Diem 2 persons 5 days \$21.50/day	<u>215</u>
	2,776
35% OH	<u>972</u>
	\$3,748

COMPLAINT 10-1073-04
MICH. TECH. UNIV. / WANDLISH

Date OCT. 17, 1984

Page No. 1

ON OCTOBER 16th, THE UNDERSIGNED OFFICER WENT AND DID A FOLLOW UP INVESTIGATION ON THE ATTACHED COMPLAINT. THE UNDERSIGNED WAS MET AT THE SHERIFF DEPARTMENT OFFICE BY PETE CATTELINO RESEARCH ASSOCIATE FOR THE ELF ECOLOGICAL MONITORING PROGRAM WHICH IS BEING CONDUCTED BY MICHIGAN TECH UNIVERSITY, FOREST DEPARTMENT.

HOWAK (INVESTIGATING OFFICER) ALONG WITH CATTELINO, WENT OUT TO THE RESEARCH SITE (SEE ATTACHED MAP) WHICH IS LOCATED NEAR THE PAINT POND AREA, TO INVESTIGATE A MALICIOUS DESTRUCTION COMPLAINT.

UPON ARRIVAL IT WAS NOTED THAT A SOLAR COLLECTOR PANEL WAS REMOVED FROM IT'S BRACKET. THE PANEL, BRACKET AND ATTACHED ASSEMBLY WAS CHECKED FOR TOOL MARKS AND DUSTED FOR LATENT PRINTS. NO TOOL MARKS COULD BE FOUND ON THE UNIT, ONE PARTIAL FINGER PRINT AND ONE PARTIAL PALM PRINT WERE FOUND AND LIFTED WITH FINGER PRINT LIFT TAPE. THE PARTIAL PRINTS ARE NOT ENOUGH TO SEND INTO THE STATE AND FEDERAL GOVERNMENT TO ATTEMPT AN IDENTIFICATION SEARCH WITHOUT HAVING A SUSPECT WITH PRINTS ON FILE.

AFTER THE UNIT WAS EXAMINED THE CABLE AND WIRE SYSTEMS WERE CHECKED. IT WAS NOTED THAT THE CABLES AND WIRES WERE CUT, POSSIBLY WITH A KNIFE, WHICH LEFT THE COPPER SHIELD ON THE CABLE EXPOSED TO THE ELEMENTS. THE ENTIRE CABLE SYSTEM HAD NUMEROUS CUTS IN VARIOUS SPOTS WHICH INDICATED THAT WHOEVER DID THE DAMAGE DID NOT DO SO SYSTEMATICLY BUT CHOSE RANDOM AREAS ON THE CABLES. THE ENTIRE CABLE SYSTEM APPEARED TO BE IN WORKING ORDER. AT VARIOUS LOCATIONS THE CABLE WAS DUSTED FOR LATENT PRINTS, NONE COULD BE FOUND.

ONE SET OF WIRES WHICH WERE ATTACHED TO THE GROUND SENSORS WAS MISSING, THE AREA WAS SEARCHED IN AN ATTEMPT TO RECOVER THE WIRES BUT THEY COULD NOT BE FOUND. SOME OF THE OTHER WIRES WHICH ARE ATTACHED TO THE GROUND SENSORS WERE CUT THE SAME AS THE CABLE SYSTEM WAS, THIS BEING THAT THE INSULATING JACKETS WERE CUT AWAY.

ONE SET OF GROUND SENSORS LOCATED TO THE SOUTH WERE PULLED OUT OF THE GROUND. THE SENSORS WERE NOT REMOVED FROM THE RESEARCH SITE, BUT LEFT ON THE GROUND WHERE THEY CONTINUED.

COMPLAINT # 10-1078-84
rich.tech.univ./vandalism

Date CONTINUED Page No. 2

CONTINUED:(SENSORS) HAD BEEN PULLED FROM THE GROUND.THE GROUND SENSORS
COULD NOT BE DUSTED FOR LATENT PRINTS DUE TO THE FACT THAT THEY ARE MADE WITH
A ROUGH TEXTURED FINISH AND PRINTS COULD NOT BE LOCATED.

A RAIN GAUGE WHICH IS LOCATED NEAR THE TRANSMITTER UNIT WAS PULLED FROM
THE GROUND,THIS WAS ALSO DUSTED FOR LATENT PRINTS BUT NONE COULD BE FOUND.

THE PLOT STAKES AND MARKER FLAGS WERE REMOVED,MOST OF THEM WERE PILED
NEAR TREES,SOME WERE TOSSED INTO A LITTER TRAP WHICH WAS USED IN THE RESEARCH
PROJECT TO COLLECT LEAVES,TWIGS, SEED ETC.TWO LITTER TRAPS WERE MOVED DISTURBING
THE CONTENTS AND ALSO ONE OF THE TRAPS WAS OVERTURNED. SOME OF THE OTHER TRAPS
ALSO HAD STAKES AND FLAGS TOSSED INTO THEM.

DENDROMETER BANDS WHICH ARE USED TO MEASURE TREE GROWTH HAD BEEN REMOVED.
THE BANDS APPEARED TO HAVE BEEN CUT OFF,SOME OF THESE BANDS WERE TOSSED INTO LITTER
TRAPS ,MOST OF THEM WERE LEFT ON THE GROUND,SOME OF THE BANDS WERE DUSTED FOR
LATENT PRINTS BUT NONE COULD BE FOUND.IT APPEARED THAT THE DAMAGE WAS DONE BY MORE THAN
ONE PERSON AND ALSO THAT GLOVES MUST HAVE BEEN WORN ESPECIALLYWHILE REMOVING
THE BANDS BECAUSE THE BANDS HAVE A VERY SHARP EDGE AND IT WOULD BE LIKELY THAT
SOMEONE WOULD HAVE CUT THEMSELVES.NO BLOOD COULD BE FOUND ON ANY OF THE BANDS.

THE AREA WAS SEARCHED FOR ANY PHYSICAL EVIDENCE BUT NONE COULD BE
FOUND. PHOTOS WERE TAKEN BY PETE CATTELINO WHILE NOIAK CONDUCTED HIS INVESTIGATION.

WHILE LEAVING THE AREA TWO ORANGE MARKER FLAGS WERE FOUND.THE FLAGS HAD
WRITTING ON THEM. ONE FLAG HAD WRITTEN ON IT "GO STOP THEM PROJECT ELF SEA FAKER"
THE OTHER HAD WRITTEN ON IT "ELF TERMINAL SITE 1/4 MILE". CATTELINO ADVISED NOIAK
THAT THE FLAGS THEMSELVES DID NOT BELONG TO THE PROJECT.

ALSO ONE FLAG THAT DID BELONG TO THE PROJECT HAD BEEN WRITTEN ON.ON THIS
FLAG SOMEONE HAD WROTE PROJECT ELF.

ON THE PAINT POND ROAD A PAPER SIGN WAS FOUND UPON THIS SIGN THERE WAS

CONTINUED

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best available copy.

COMPLAINT # 10-1073-04
HIGH TECH. UNIV. / VANDALISM

Date CONTINUEDPage No 3

CONTINUED: AN ARROW POINTING TOWARD THE DIRECTION OF THE RESEARCH PROJECT,
ALSO WRITTEN ON THE SIGN WAS "PROJECT ELF TERMINAL SITE". THE FLAGS AND THE SIGN
WERE TAKEN AND TAGGED AS EVIDENCE. NOWAK RETURNED TO THE SHERIFF DEPARTMENT.

NOWAK REQUESTED THAT CATTELINO SEND INFORMATION TO NOWAK WITH MAPS AND
DIAGRAMS IN REFERENCE TO THE DAMAGE. CATTELINO ALSO ADVISED NOWAK THAT THE
DOLLAR VALUE OF THE DAMAGE AND LOSS OF RESEARCH IS APPROX: \$30,000.00 .

OCTOBER 26, 1984.

ON 10-26-84 NOWAK WAS CALLED TO THE SHERIFF OFFICE TO MEET WITH
LT. CHARLES WALKER OF THE UNITED STATES NAVY, WHO IS THE PROJECT MGR. IN THE
AREA FOR PROJECT ELF. NOWAK SPOKE WITH WALKER AND HAD COPIES OF THE REPORTS
MADE FOR WALKER.

WALKER AND NOWAK THEN WENT OUT TO THE RESEARCH SITE WHERE NOWAK
AND WALKER REVIEWED THE INVESTIGATION . WALKER TOOK PHOTOS OF THE AREA.

OCTOBER 27, 1984

ON 10-27-84 NOWAK WENT TO THE TURVEY HOME LOCATED IN IRON COUNTY.
CHARLES TURVEY AND HIS WIFE JO, ARE ORGANIZERS OF A GROUP IN THE IRON COUNTY
AREA WHICH IS KNOWN AS C.A.T.E. (CITIZENS AGAINST TRIDENT ELF). THE TURVEYS HAVE
ADMITTED TO NEWS MEDIA THAT IN THE PAST THEY HAVE DISRUPTED SURVEY STAKES BY
PULLING THEM OUT OF THE GROUND. NOWAK QUESTIONED THE TURVEYS ABOUT THE RESEARCH
SITE INCIDENT. BOTH OF THE TURVEYS STATED THAT THEY DID NOT HAVE ANY KNOWLEDGE
OF ANY PROJECT SITE WITHIN IRON COUNTY, AND ALSO THAT THEY DID NOT HEAR ANY
INFORMATION AS TO WHO THE RESPONSIBLE PEOPLE ARE. MR. TURVEY FURTHER STATED
THAT IF HE DID KNOW HE WOULDN'T TELL ME, AND THAT IT IS NOTHING AGAINST ME
BUT THAT HE DOES NOT BELIEVE IN HELPING ANYONE IN REFERENCE TO THE INVESTIGATION.

CONTINUED

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best available copy.

COMPLAINTS 10-1073-04

Date CONTINUEDPage No. 4 131

CONTINUED: MR. AND MRS. TURVEY STATED THAT THEY WOULD LIKE TO MEET WITH
CAPT. KOSITZ OR LT. WALKER. NOIAK ADVISED THEM THAT HE (NOIAK) WOULD RELAY THE
REQUEST TO LT. WALKER. THE TURVEYS STATED THAT THE GROUP IS NOT A VIOLENT GROUP
BUT IF THEY HAD TO GO TO JAIL THEY WOULD BECAUSE THEY STRONGLY BELIEVE THAT THE
ELF PROJECT SHOULD BE STOPPED. BOTH TURVEYS WERE NOT HOSTILE TOWARD THE REPORTING
OFFICER. THE TURVEYS GAVE THE ANTI ELF GROUPS NEWS LETTER TO THE REPORTING OFFICER
AND MR. TURVEY STATED THAT HE DOES NOT OWN A GUN AND IS NOT A VIOLENT PERSON. TURVEY
FURTHER STATED THAT THE GROUP WOULD NOT BLOW UP ANY TRANSMITTER SITE NOR WOULD THEY
DO ANY PHYSICAL DAMAGE TO THE ELF PROJECT, BUT THAT THEY WILL CONTINUE TO PULL OUT
ANY ELF SURVEY STAKES THAT THEY CAN FIND. THE TURVEYS STATED THAT THEY ARE TOTALLY
AGAINST ANY THING TO DO WITH NUCLEAR WEAPONS AND THE ELF PROJECT AND THAT THEY
HAVE MOVED THIS AREA BY CHOICE AND THAT THEY FEEL THAT PROJECT ELF WILL HARM THE
ENVIRONMENT. NOIAK LEFT THE TURVEY RESIDENCE AND RETURNED TO PATROL DUTIES.

OCTOBER 28, 1984

NOIAK CONTACTED LT. WALKER BY PHONE AND ADVISED HIM THAT THE TURVEYS
CLAIMED NO KNOWLEDGE OF THE RECENT INCIDENT, AND THAT EVEN IF THEY DID THAT THE
TURVEYS WOULD NOT COOPERATE. NOIAK DISCUSSED THE INVESTIGATION WITH WALKER
AND THEN ENDED THE CONVERSATION.

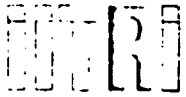
NOIAK THEN PHONED THE TURVEY HOME AND TALKED WITH CHARLES TURVEY,
AND ADVISED HIM THAT LT. WALKER WAS GIVEN TURVEYS REQUEST, AND QUESTIONED TURVEY ABOUT
THE INCIDENT BUT TURVEY DECLINED TO ANSWER ANY OF THE QUESTIONS. NOIAK THEN
ENDED THE CONVERSATION WITH TURVEY.

DEPUTY FRANK V. NOIAK

100th COUNTY SHERIFF DEPT.

APPENDIX B

Summary of Electromagnetic field measurements



IIT Research Institute
10 West 35 Street, Chicago, Illinois 60616
312/567-4000

29 November 1984

Glen Mroz, Ph.D
Department of Forestry
Michigan Technological University
Houghton, MI 49931

Dear Glen:

This letter is a summary of electromagnetic (EM) field measurements taken at your study sites. Please review and comment, if you wish.

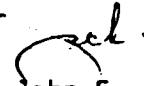
On 15 and 21 May, personnel from the IIT Research Institute made EM field measurements to assist in your final selection of study sites. EM field intensities, comparison of data to exposure criteria, and site locations were detailed in a 27 June 1984 letter. Subsequently, sites 4C1, 4T2 and 4T4 were remeasured on 6 August and 9 August.

A summary of measured 60 Hz data for your study sites are listed in Table 1. Estimated 76 Hz data was detailed in our 27 June letter. Both 1983 and 1984, 60 Hz data are provided for preliminary indications of exposure variability. Several new measurement points at your control site (4C1-6, -7, -8 and -9) were established to determine the spatial variability of 60 Hz fields from a nearby transmission line. The location of these measurement points are detailed on Figure 1. Fewer points were required to characterize your test plots as they are remote from electric power distribution systems. Measurement point 4T2-3 is located in the northeast corner of the cleared plots at stake number 1, while measurement point 4T4-4 is in the northwest corner of the cleared plot at stake number 2. Measurement points 4T4-2 and 4T4-3 are 3 meters north and southeast of the GOES transmitter respectively. These later two points were occupied to determine EM fields strengths in the vicinity of the transmitter.

All of your sites will be remeasured during 1985. If available, please forward plot maps for sites 4T2 and 4T4. Your comments on the required number of measurement points for each site, or other inputs, will be appreciated.

Sincerely,

IIT RESEARCH INSTITUTE



John E. Zapotosky

JEZ/bjm

cc: RBrosh
RDCarlson/File
JRGauger

Table 1
Electric Field Intensities and
Magnetic Flux Densities¹

Site No.	Meas. Pt.	Meas. Yr.	Transverse E Field (Air) (V/M)		Longitudinal E Field (Earth) (mV/M)		Magnetic Flux Density (mG)	
			76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz
4C1	1	83	--	<0.001	--	0.27	--	0.011
4C1	1	84	--	--	--	0.29	--	0.008
4C1	2	83	--	<0.001	--	0.56	--	0.18
4C1	3	83	--	<0.001	--	0.004	--	0.001
4C1	3	84	--	--	--	--	--	0.001
4C1	4	83	--	--	--	0.16	--	0.009
4C1	5	83	--	--	--	0.38	--	0.005
4C1	5	84	--	--	--	0.18	--	0.002
4C1	6	84	--	0.003	--	0.020	--	0.003
4C1	7	84	--	0.006	--	0.14	--	0.003
4C1	8	84	--	0.004	--	0.10	--	0.003
4C1	9	84	--	<0.001	--	0.010	--	0.003
4T2	1	83	--	<0.001	--	0.24	--	<0.001
4T2	2	83	--	--	--	0.25	--	<0.001
4T2	3	84	--	0.001	--	0.51	--	0.002
4T4	1	84	--	<0.001	--	0.46	--	0.002
4T4	2	84	--	0.003	--	0.35	--	0.003
4T4	3	84	--	0.001	--	0.59	--	0.004
4T4	4	84	--	0.003	--	0.72	--	0.004

1 Values shown are magnitudes determined as the square root of the sum of the squares of the orthogonal field components measured. Data listed for 76 Hz is estimated based on analyses using the proposed location and operating conditions of the antenna elements along with the distance to each measurement point.

ELF Control Plots.

(Paint Pond Road)

SW 1/4, SW 1/4, Sec. 3

T41N - R32W

Figure 1 - ELF Ecological Monitoring Progr
Trees and Herbaceous Plants Stu
Michigan Technological Universi



Total Research Need

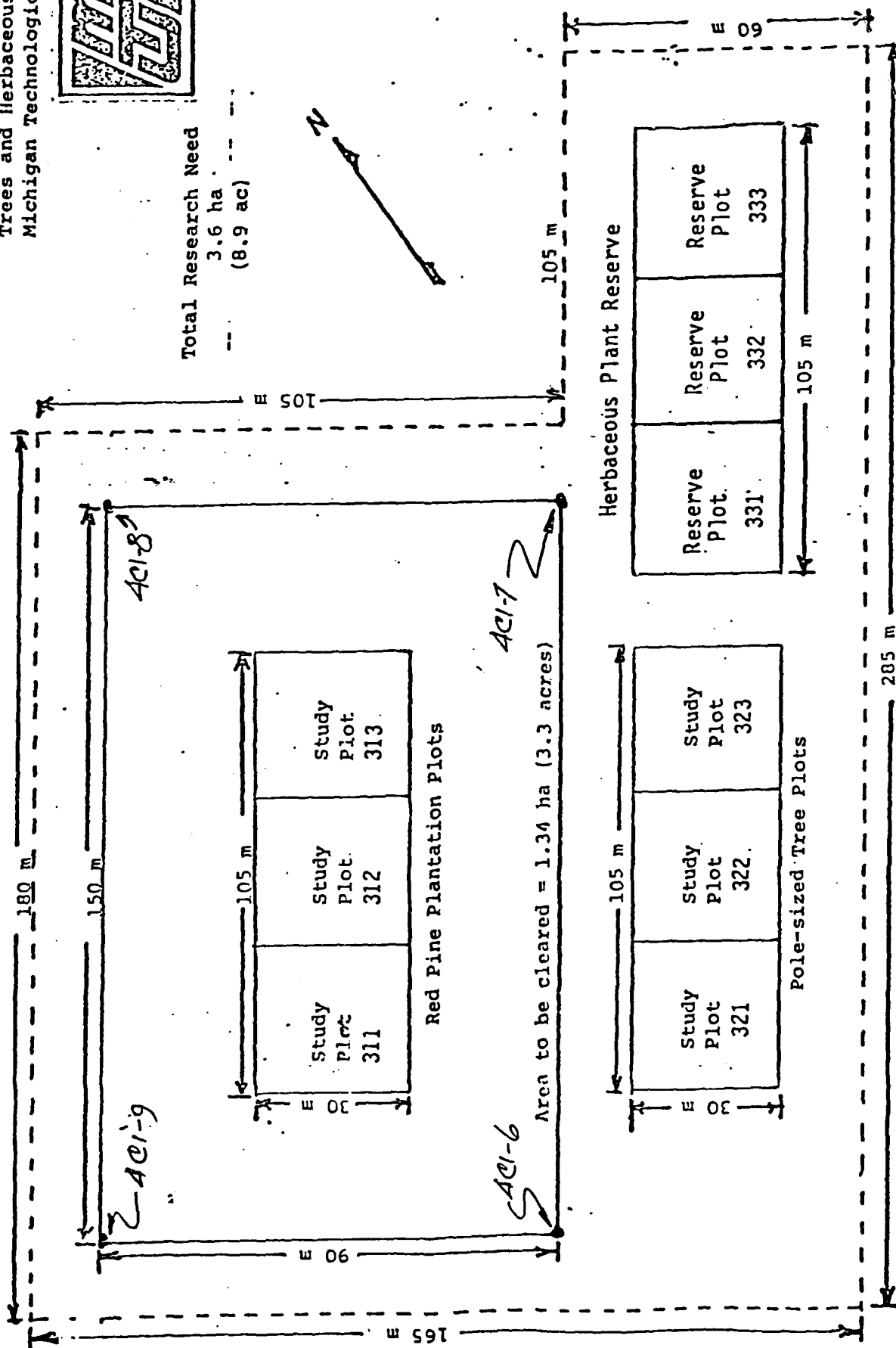
3.6 ha

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138



APPENDIX C

Equations used in determining
whole tree biomass.

Equations used in determining whole tree biomass.

<u>Species</u>	<u>Equation (Author)</u>
Bigtooth Aspen ¹	$Y = .34LN [.6800+2.2234LN(DBH)+.3390LN(HT)]$ (Young <u>et al.</u> 1964)
Hard Maple ²	$LogY = 3.186+2.35 Log(DBH)$ (Ribe, 1979)
Jack Pine ³	$LogY = 1.0368+2.4206 Log(DBH)$ (Hegyi, 1972)
Northern Red Oak ¹	$LN Y = (2.57 * LN (DBH))+.82$ (Burks, 1981)
Paper Birch ¹	$Y = .42LN [.8025+2.2234LN(DBH)+.3390LN(HT)]$ ¹ Young <u>et al.</u> 1964)
Quaking Aspen ³	$LogY = -1.1115+2.3466 Log(DBH)$ (Peterson <u>et al.</u> 1970)
Red Maple ²	$LogY = 3.033+2.466 Log(DBH)$ (Ribe, 1979)
Red Pine ¹	$LN Y = 0.8345622+2.4185 LN(DBH)$ (Winsauer and Steinhilb, 1980)
White Pine ¹	$Y = .36LN [.6592+2.2234LN(DBH)+.3390LN(HT)]$ (Young <u>et al.</u> 1964)

¹Y is ODW in pounds, DBH is diameter breast height in inches and ht is height in feet.

²Y is ODW in grams, DBH is diameter breast height in inches.

³Y is ODW in Kilograms, DBH is diameter breast height in centimeters.

TOTAL BIOMASS BY 1 CM. DIAMETER CLASSES
BIOMASS IN KILOGRAMS
ANTENNA - 1984

DBH	NRO	PB	BTA	RM	TOTAL
10	000.00	000.00	000.00	241.91	241.91
11	40.50	000.00	000.00	867.81	908.31
12	113.95	000.00	000.00	658.50	772.45
13	147.95	63.43	42.21	668.17	925.76
14	244.03	73.16	51.15	1240.83	1609.17
15	301.75	000.00	000.00	1304.98	1606.73
16	000.00	000.00	000.00	1514.79	1514.79
17	545.53	112.35	000.00	2010.44	2668.32
18	334.18	000.00	000.00	796.86	1131.04
19	365.78	303.85	000.00	466.93	1136.56
20	1062.28	184.10	120.88	689.27	2056.53
21	000.00	000.00	000.00	798.93	718.93
22	768.99	000.00	144.55	437.86	1351.38
23	883.26	000.00	000.00	000.00	883.26
24	960.79	000.00	579.36	000.00	1540.15
25	730.93	000.00	195.85	294.49	1221.28
26	000.00	000.00	448.69	000.00	448.69
27	891.28	000.00	000.00	363.45	1254.73
28	000.00	394.18	258.13	000.00	652.32
29	1066.40	410.35	290.13	000.00	1766.88
30	1771.14	000.00	620.33	000.00	2391.47
31	633.24	000.00	000.00	000.00	633.24
32	1413.65	000.00	000.00	000.00	1413.65
33	755.67	000.00	400.80	000.00	1156.46
34+	5643.65	000.00	000.00	000.00	5643.65
<hr/>					
TOTAL	18674.93	1541.43	3156.43	12355.20	35727.99

NRO - NORTHERN RED OAK
PB - PAPER BIRCH
BTA - BIG TOOTH ASPEN
RM - RED MAPLE

TOTAL BIOMASS BY 1 CM. DIAMETER CLASSES
BIOMASS IN KILOGRAMS
CONTROL - 1984

DBH	NRO	PB	BTA	RM	QA	TOTAL
10	694.11	184.87	000.00	121.73	000.00	1004.71
11	267.71	562.53	000.00	331.29	000.00	1161.53
12	1141.16	264.23	000.00	167.26	000.00	1572.65
13	2355.23	426.38	000.00	000.00	000.00	2781.61
14	3332.79	520.07	000.00	000.00	000.00	3852.85
15	3004.32	472.08	000.00	000.00	172.17	3648.57
16	2558.94	397.45	86.64	000.00	79.41	3122.46
17	962.28	698.41	404.21	000.00	000.00	2064.89
18	1755.19	98.34	000.00	000.00	140.71	1994.23
19	3701.50	531.50	706.02	000.00	465.61	5404.62
20	3734.20	160.81	347.15	000.00	86.38	4328.53
21	4644.72	000.00	243.80	000.00	165.84	5054.36
22	2055.94	000.00	91.08	000.00	000.00	2147.01
23	1831.13	131.95	406.94	000.00	154.62	2524.64
24	2617.84	84.04	134.46	000.00	222.15	3058.48
25	2233.58	126.17	289.41	000.00	41.76	2690.92
26	1727.38	46.50	744.12	000.00	253.77	2771.77
27	2054.48	000.00	147.58	000.00	131.45	1217.80
28	483.93	000.00	733.87	000.00	000.00	1217.80
29	681.73	000.00	000.00	000.00	000.00	681.73
30	174.18	000.00	000.00	000.00	000.00	174.18
31	87.42	000.00	000.00	000.00	127.59	215.01
32	433.23	000.00	000.00	000.00	000.00	433.29
33	964.93	000.00	000.00	000.00	000.00	964.92
34+	595.34	000.00	000.00	000.00	000.00	595.34
<hr/>						
TOTAL	44511.69	4754.32	4515.54	657.54	2110.613	56549.70

NRO - NORTHERN RED OAK
PB - PAPER BIRCH
BTA - BIG TOOTH ASPEN
RM - RED MAPLE
QA - QUAKING ASPEN

APPENDIX D

Soil Profile Description and
Compositional Analysis of the
Soil Occurring on the Control
Study Area.

Fedon Classification: Alfic Paleorthod; coarse-loamy, mixed, frigid.

Series Classification: (Cose)

Soil:

Plot and Study: ELF (Control Site)

Location: Iron County, Michigan. SF, SF Section 3, T41N, R32W.

Climate: Average annual precipitation is about 850 mm; mean annual air temperature is about 8°C.

Vegetation and Land Use: Woodland (Red oak, white birch, aspen, sugar maple).

Parent Material: Glacial till.

Physiographic Position: Rolling upland.

Topography: Complex slopes. Gradient is 3 to 5 percent. Southeast aspect. Concave, upper slope position. Slope length is 30 meters.

Groundwater: Below 230 cms.

Sampled by: R. Wendell, E. Wilczynski. September 20, 1984.

(All colors are for moist conditions.)

O1 548 5 to 2 cm. Undecomposed hardwood leaves and twigs; very strongly acid; abrupt smooth boundary. (2 to 3 cm thick)

Oe 549 2 to 0 cm. Partially decomposed hardwood litter; very strongly acid; abrupt smooth boundary. (0 to 2 cm thick)

A 550 0 to 4 cm. Dark reddish brown (5YP 2.5/2) fine sandy loam; weak fine granular structure; very friable; many fine roots; extremely acid; clear smooth boundary. (2 to 5 cm thick)

E 551 4 to 9 cm. Pinkish gray (5YP 6/2) fine loamy sand; weak fine subangular blocky structure; friable; many fine and common medium roots; extremely acid; clear wavy boundary. (5 to 9 cm thick)

Pt1 552 9 to 32 cm. Yellowish red (5YP 4/6) fine loamy sand; moderate medium subangular blocky structure; friable; many fine, common medium and few coarse roots; 3 percent pebbles; medium acid; gradual smooth boundary. (19 to 23 cm thick)

Pt2 553 32 to 55 cm. Yellowish red (5YP 5/8) fine sand; strong medium subangular blocky structure; friable; few fine and many medium roots; 4 percent pebbles; slightly acid; clear smooth boundary. (20 to 23 cm thick)

E1 554 55 to 67 cm. Reddish brown (5YP 5/3) fine sandy loam; moderate medium subangular blocky structure; friable; few medium roots; few fine vesicular pores; 9 percent pebbles; medium acid; gradual smooth boundary. (12 to 14 cm thick)

(E/E)1 555 67 to 105 cm. Reddish brown (5YP 4/4) gravelly fine sandy loam (Pt) and light reddish brown (5YP 6/4) fine loamy sand (E); strong fine subangular blocky structure (Pt) and strong medium subangular blocky structure (E); friable; few fine and few medium roots; few fine vesicular pores; 34 percent pebbles; medium acid; gradual smooth boundary. (38 to 40 cm thick)

(E/E)2 556 105 to 124 cm. Red (2.5YP 4/6) sandy loam (Pt) and yellowish red (5YP 5/6) loamy sand (E); strong medium subangular blocky structure; friable; few fine roots; few very fine vesicular pores; 13 percent pebbles; slightly acid; clear smooth boundary. (17 to 19 cm thick)

C 557 124 to 230 cm. Yellowish red (5YP 5/6) sand; single grain; loose; 8 percent pebbles; slightly acid; few irregularly spaced red (2.5YP 4/6) loamy sand bands.

NOTE: A layer with 70 percent pebble content occurred between 89 and 109 cm.

APPENDIX E

Analyses of the Composite Soil
Samples from the Control Plot

APPENDIX F

Configuration of the Ambient Monitoring Platforms

CONTROL PLATFORM

<u>Plot</u>	<u>Channel</u>	<u>Sensor</u>	<u>Probe Number</u>	
321	1	Air Temperature		Not in Use*
	2	Soil Temperature 5 cm	138	
	3	Soil Moisture 5 cm		
	4	Soil Temperature 10 cm	139	
	5	Soil Moisture 10 cm		
322	6	Air Temperature		
	7	Soil Temperature 5 cm	140	
	8	Soil Moisture 5 cm		
	9	Soil Temperature 10 cm	141	
	10	Soil Moisture 10 cm		
	11	Soil Temperature 30 cm	142	Not in Use
	12	Soil Moisture 30 cm		
	13	Soil Temperature 100 cm	154	Not in Use
	14	Soil Moisture 100 cm		
	15	Soil Temperature 200 cm		
	16	Soil Moisture 200 cm		
323	17	Air Temperature		Not in Use
	18	Soil Temperature 5 cm	143	
	19	Soil Moisture 5 cm		
	20	Soil Temperature 10 cm	144	
	21	Soil Moisture 10 cm		
311	22	Air Temperature		Not in Use
	23	Soil Temperature 5 cm	145	
	24	Soil Moisture 5 cm		
	25	Soil Temperature 10 cm	146	
	26	Soil Moisture 10 cm		
312	27	Air Temperature		
	28	Soil Temperature 5 cm	149	
	29	Soil Moisture 5 cm		
	30	Soil Temperature 10 cm	150	
	31	Soil Moisture 10 cm		
313	32	Air Temperature		
	33	Soil Temperature 5 cm	152	
	34	Soil Moisture 5 cm		
	35	Soil Temperature 10 cm	153	
	36	Soil Moisture 10 cm		
	37	Precipitation		
	38	Relative Humidity		
	39	Voltage		
	40	Snow Pillow		

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*Waiting MIT board modifications.

GROUND PLATFORM

<u>Plot</u>	<u>Channel</u>	<u>Sensor</u>	<u>Probe Number</u>	
111	1	Air Temperature		
	2	Soil Temperature 5 cm	131	
	3	Soil Moisture 5 cm		
	4	Soil Temperature 10 cm	132	
	5	Soil Moisture 10 cm		
112	6	Air Temperature		
	7	Soil Temperature 5 cm	133	
	8	Soil Moisture 5 cm		
	9	Soil Temperature 10 cm	134	
	10	Soil Moisture 10 cm		
	11	Soil Temperature 30 cm	135	Not in Use*
	12	Soil Moisture 30 cm		Not in Use
	13	Soil Temperature 100 cm		Not in Use
	14	Soil Moisture 100 cm		Not in Use
	15	Soil Temperature 200 cm		Not in Use
	16	Soil Moisture 200 cm		Not in Use
113	17	Air Temperature		
	18	Soil Temperature 5 cm	136	
	19	Soil Moisture 5 cm		
	20	Soil Temperature 10 cm	137	
	21	Soil Moisture 10 cm		
	22	Radiation		
	23	Relative Humidity		
	24	Voltage		
	25	Precipitation		
	26	Snow Pillow		

*Awaiting MET board modifications.

OVERHEAD PLATFORM

<u>Plot</u>	<u>Channel</u>	<u>Sensor</u>	<u>Probe Number</u>
221	1	Air Temperature	
	2	Soil Temperature 5 cm	116
	3	Soil Moisture 5 cm	
	4	Soil Temperature 10 cm	117
	5	Soil Moisture 10 cm	
222	6	Air Temperature	
	7	Soil Temperature 5 cm	118
	8	Soil Moisture 5 cm	
	9	Soil Temperature 10 cm	119
	10	Soil Moisture 10 cm	
223	11	Air Temperature	
	12	Soil Temperature 5 cm	120
	13	Soil Moisture 5 cm	
	14	Soil Temperature 10 cm	121
	15	Soil Moisture 10 cm	
211	16	Air Temperature	
	17	Soil Temperature 5 cm	122
	18	Soil Moisture 5 cm	
	19	Soil Temperature 10 cm	126
	20	Soil Moisture 10 cm	
212	21	Air Temperature	
	22	Soil Temperature 5 cm	127
	23	Soil Moisture 5 cm	
	24	Soil Temperature 10 cm	128
	25	Soil Moisture 10 cm	
213	26	Air Temperature	
	27	Soil Temperature 5 cm	129
	28	Soil Moisture 5 cm	
	29	Soil Temperature 10 cm	130
	30	Soil Moisture 10 cm	
	31	Precipitation	
	32	Relative Humidity	
	33	Voltage	
	34	Snow Pillow	

Ambient Monitoring Equipment

Variable	Equipment	Mode of Operation	Accuracy & Characteristics
Radiation	Eppley Black and White Pyranometer	Differential electroplated (copper-constantan) thermopile with balckened hot-junction receivers and cold-junction receivers whitened	<u>Spectral Range:</u> 3-3 μ m <u>Sensitivity:</u> 11 microvolts/watt meter <u>Linearity:</u> $\pm 1\%$ from 0-1400 watt/meter
Temperature (Soil and air)	VSI 44006 Thermistor	Direct relationship between electrical resistance and temperature	<u>Accuracy:</u> -30 $^{\circ}$ C to +40 $^{\circ}$ C $\pm 0.2^{\circ}$ C <u>Resolution:</u> -30 $^{\circ}$ C to +40 $^{\circ}$ C $\pm .05^{\circ}$ C
Humidity	Humicap and Handar 43.JA Conditioning unit	Hygroscopic organic layer on capacitor varies the capacitance value in proportion to moisture in air	<u>Accuracy:</u> 0 to 80% $\pm 2\%$ <u>Linearity:</u> 80% to 100% $\pm 5\%$ <u>Linearity:</u> 0 to 80% $\pm 1\%$
Soil Moisture	Hanadar 438A Soil Moisture Probe	Galvanic soil moisture sensor	<u>Range:</u> 2% to 30%
Precipitation	Tippirs Bucket	Each tip (.01 inch of water) sends a electrical pulse	<u>Accuracy:</u> under .80 inch $\pm .02$ inch over .80 less than $\pm 3\%$
Snowpillow	Viатran-218-12 Transducer and Snow Pillow	Conversion of hydraulic pressure (due to snow weight on snow pillow) convertal to voltage	<u>Error Band:</u> $\pm .4\%$ of fullscale

APPENDIX G

NESS Data Retrieval Procedure

NESS DATA RETRIEVAL PROCEDURE

Each Day

An IBM-PC is plugged into a timer which will turn it on after midnight. Once turned on, the PC runs a BASIC program that reads a file containing the date and time of the last transmission received. It then prepares a CROSSTALK script file that will collect all data from that date and time forward.

The script file is then used by the CROSSTALK communications program to dial NESS and control the collection and storage of the data to a file on the IBM-PC. After the data has been collected, another BASIC program is executed to scan the data and determine the date and time of the last transmission received. The date and time are then written to a file to be used to create the next days CROSSTALK script file.

The timer is set to turn the power off on the PC at 2:00 a.m. If the collection is successful, the PC will be sitting idle waiting to be turned off. If, however, the CROSSTALK communication program is still trying to connect with NESS, the script will be aborted. In which case, the date and time of the last transmission received remain unchanged and the next night two days of data will be collected.

The next morning, the PC is checked to determine if the data was collected. If it was, it printed and scanned for missing, partial or bad transmissions. If the data was not collected, because the PC was unable to connect with NESS, a decision is made to either collect the data that morning, or let it be collected the next night.

Once a week

The daily data collected and stored on the IBM-PC is edited and combined into a weekly data file.

The weekly data file is then run through a BASIC program that currently produces three files of summary information. One file consists of the raw data in a form suitable for inputting into the LOTUS-123 Spreadsheet program for analysis. The second file consists of the daily average, maximum and minimum values. This file is also suitable for inputting into the LOTUS-123 program for analysis. Plus, it is sent to the UNIVAC mainframe for later loading into the SIR Database. The final file contains just the daily averages. This file is sent to the UNIVAC mainframe computer for analysis by a statistical package.

The raw data, as received from NESS, is transferred to the UNIVAC mainframe computer for backup storage should the IBM-PC fail.

Once a Month

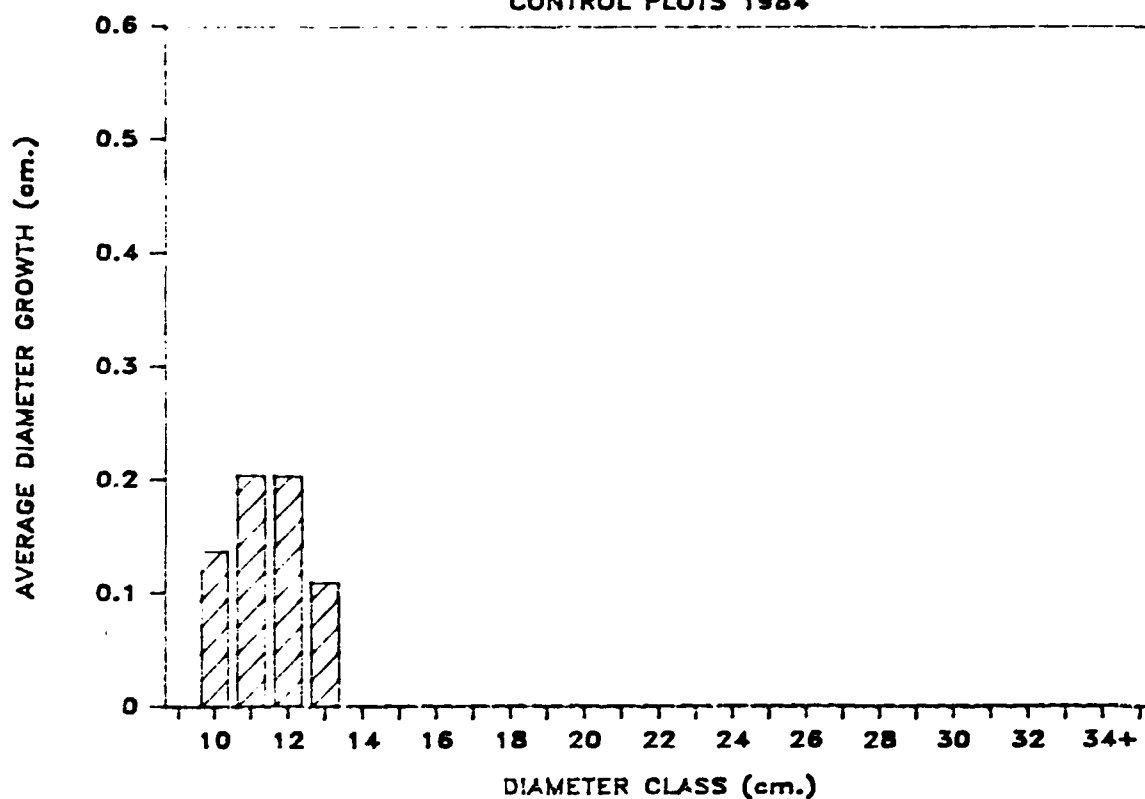
The raw data transferred to the UNIVAC is copied to tape for long term storage.

APPENDIX H

Seasonal diameter growth of each species
on the antenna and control sites.

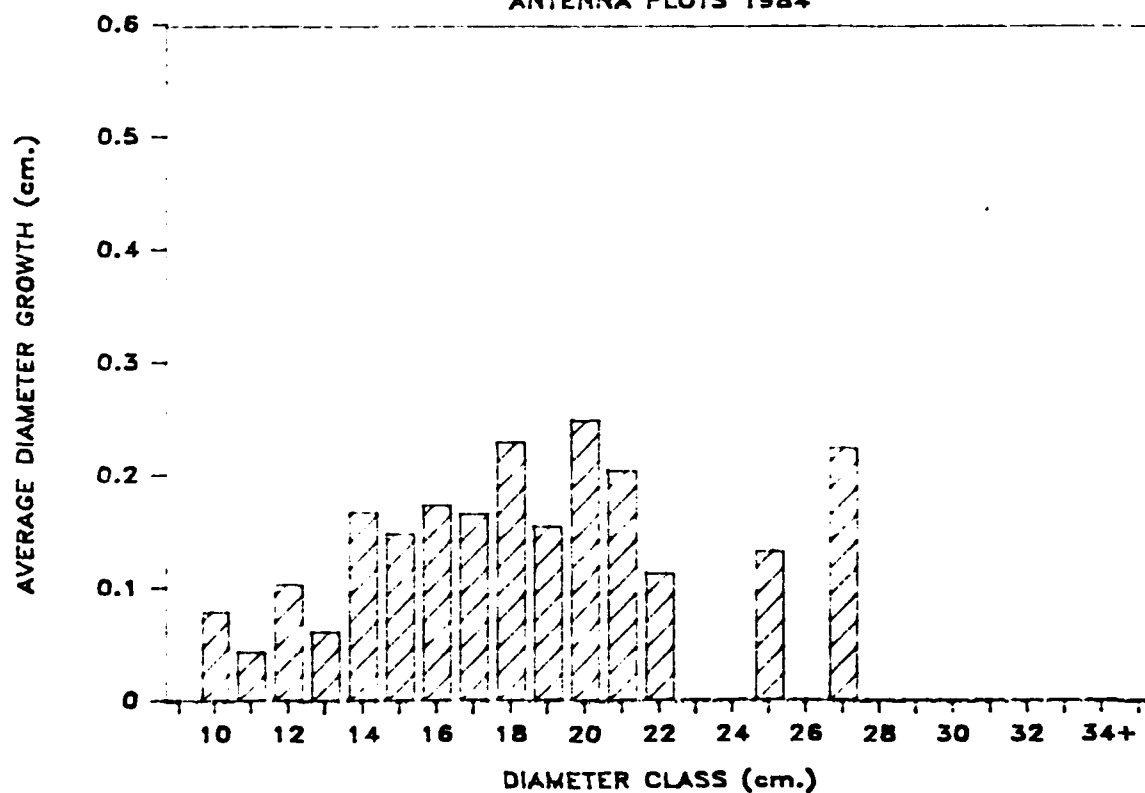
SEASONAL GROWTH — RED MAPLE

CONTROL PLOTS 1984



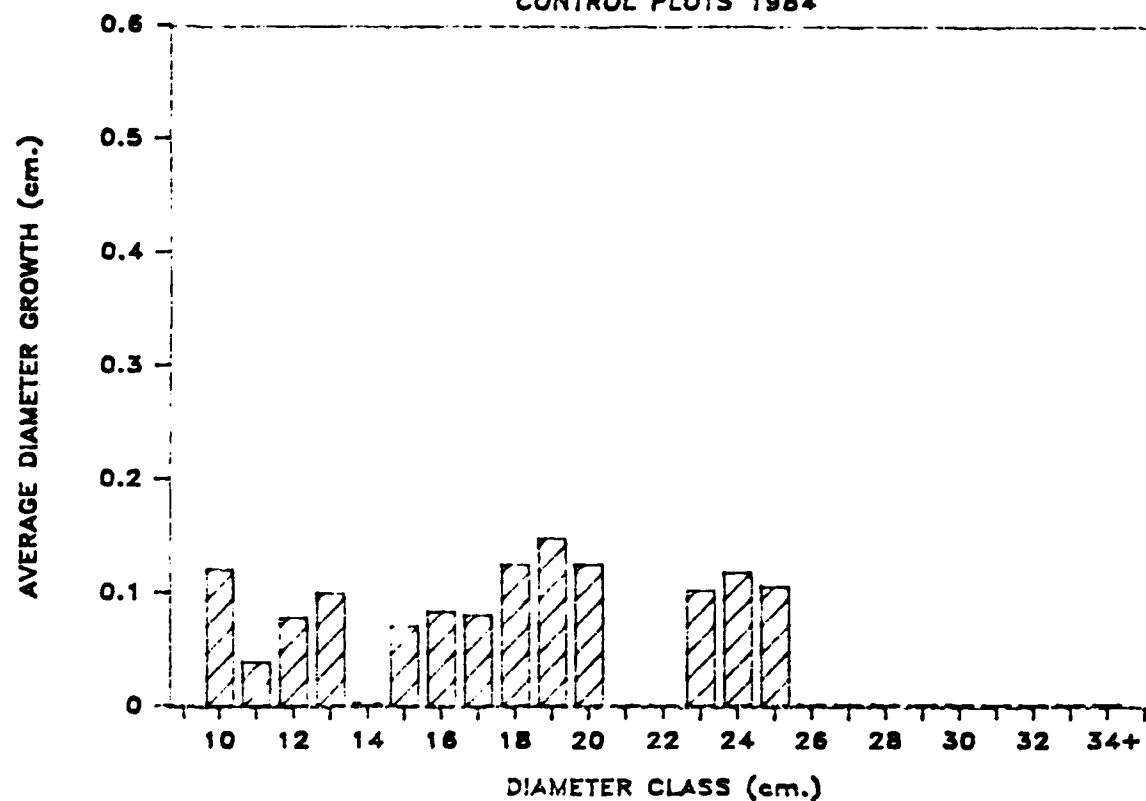
SEASONAL GROWTH — RED MAPLE

ANTENNA PLOTS 1984



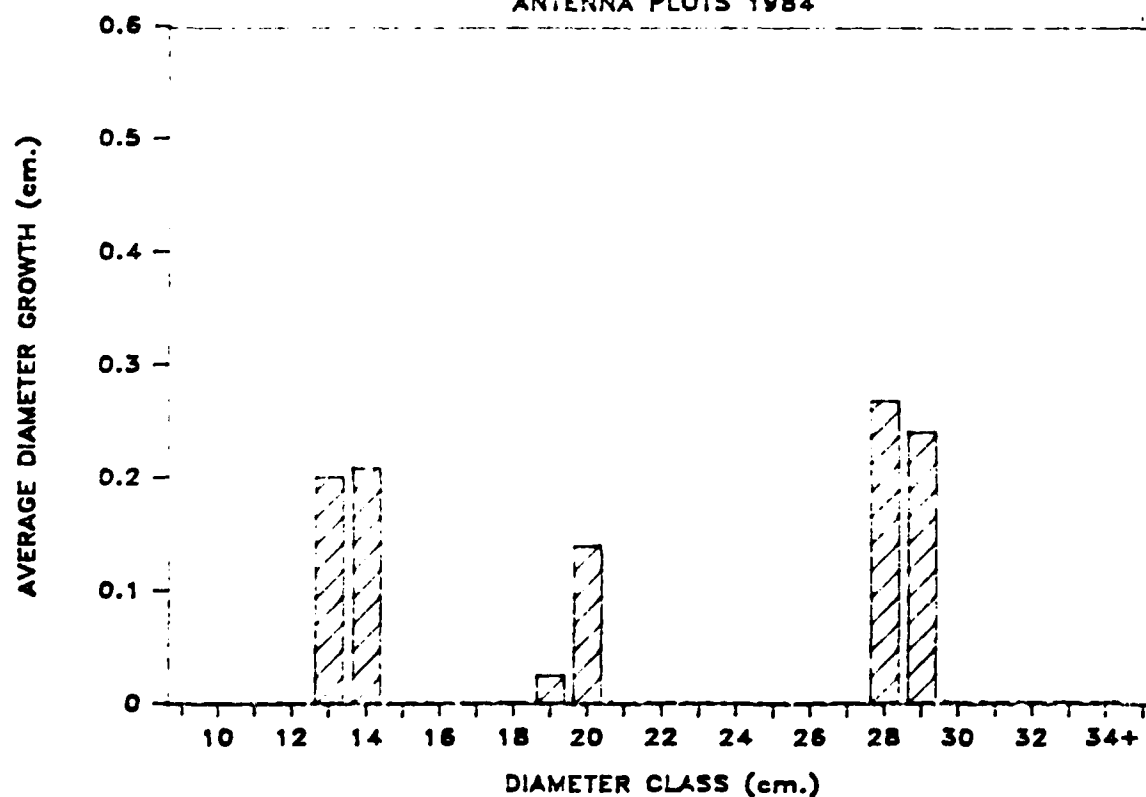
SEASONAL GROWTH — PAPER BIRCH

CONTROL PLOTS 1984



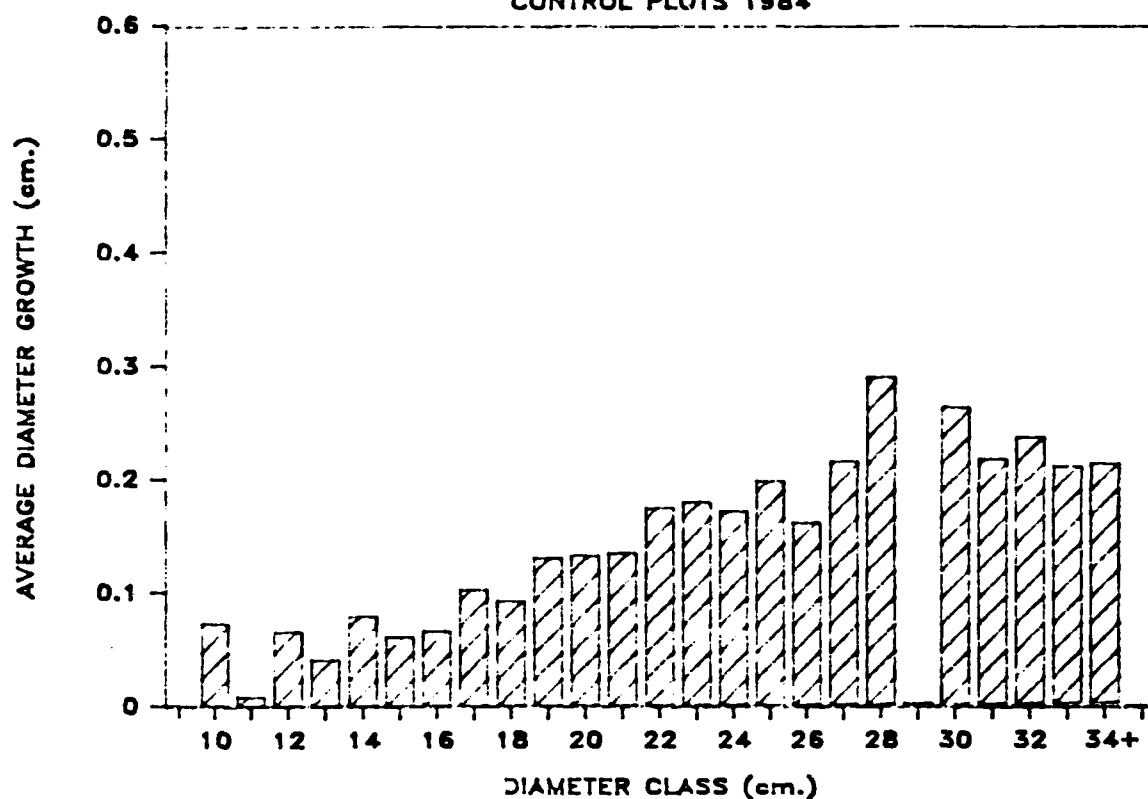
SEASONAL GROWTH — PAPER BIRCH

ANTENNA PLOTS 1984



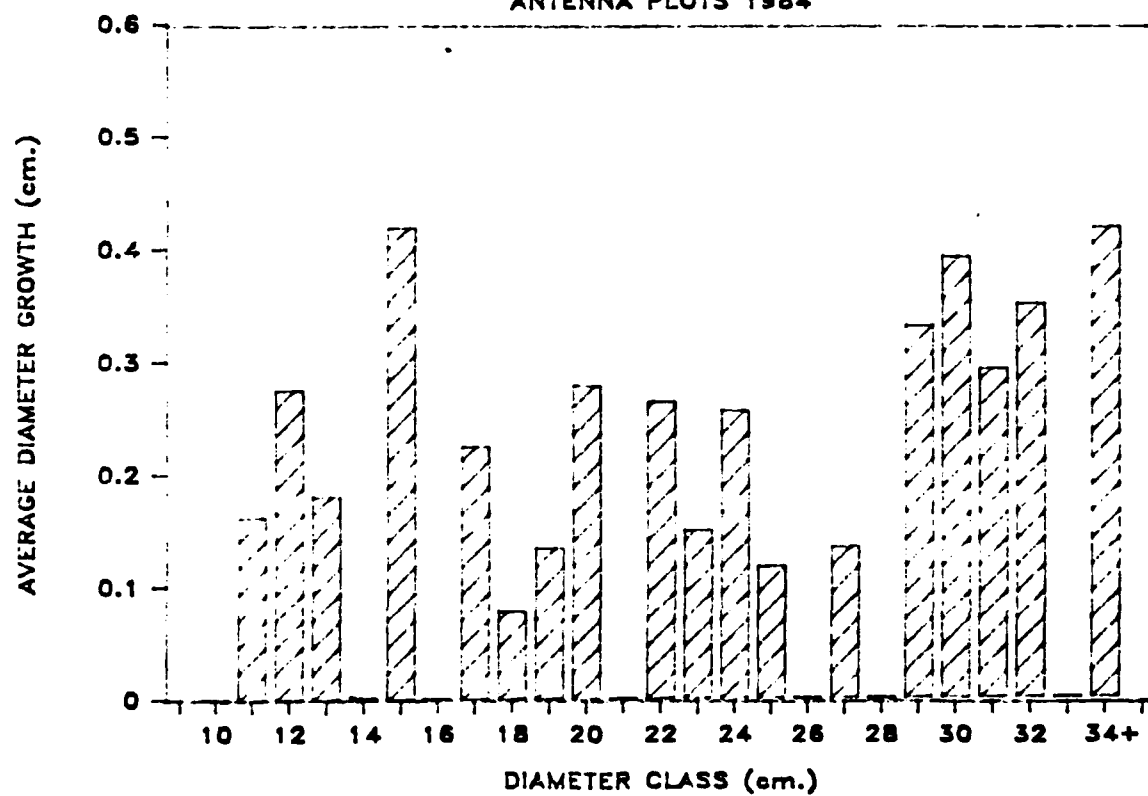
SEASONAL GROWTH — RED OAK

CONTROL PLOTS 1984



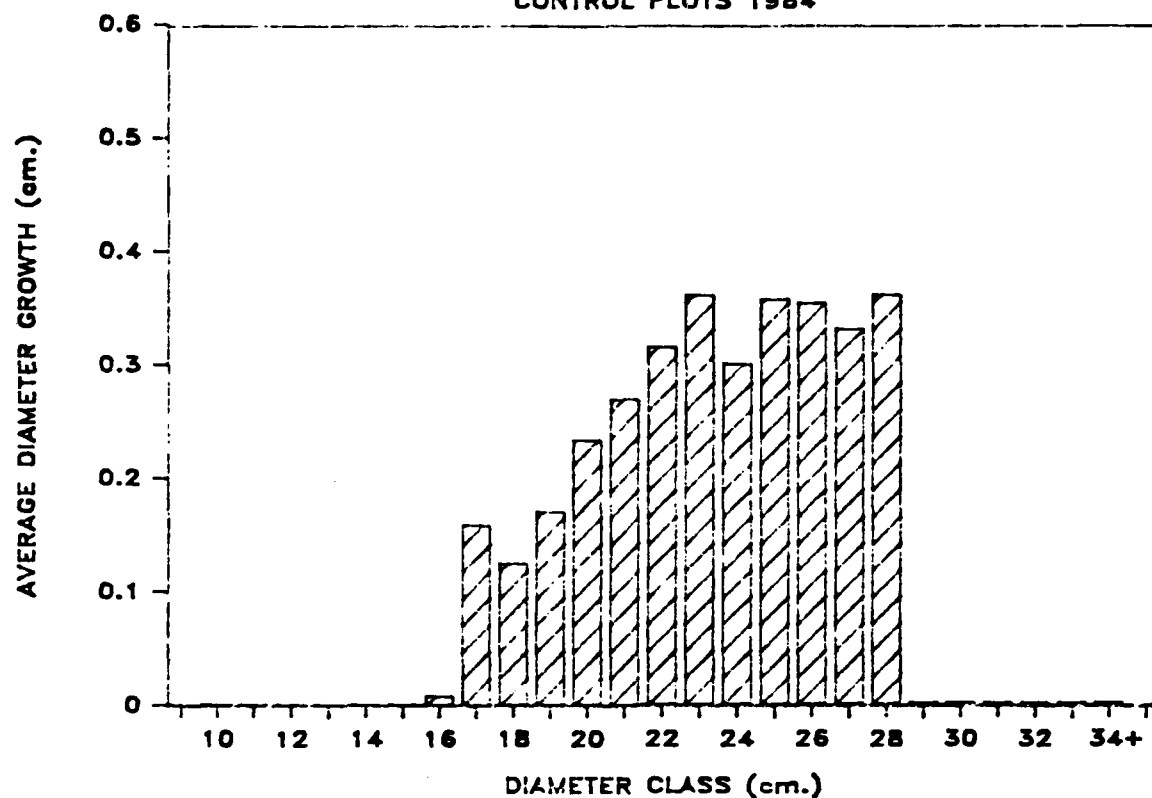
SEASONAL GROWTH — RED OAK

ANTENNA PLOTS 1984



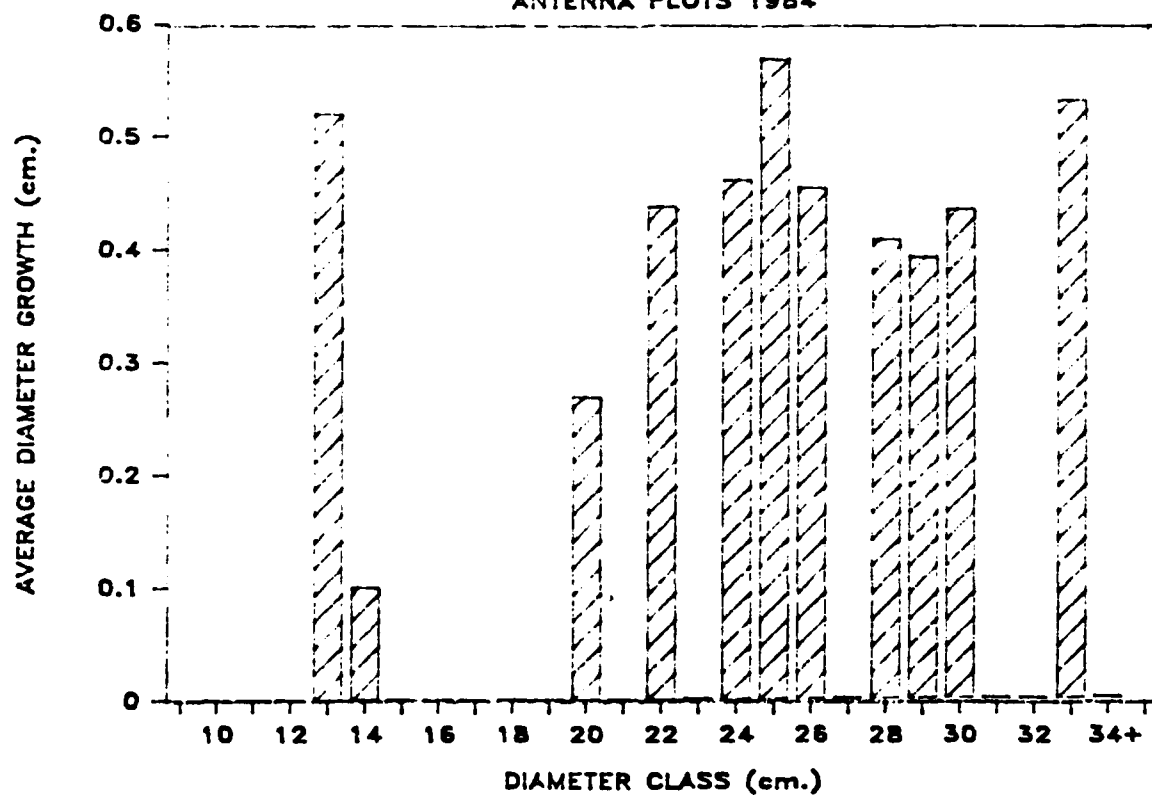
SEASONAL GROWTH — BIG TOOTH ASPEN

CONTROL PLOTS 1984



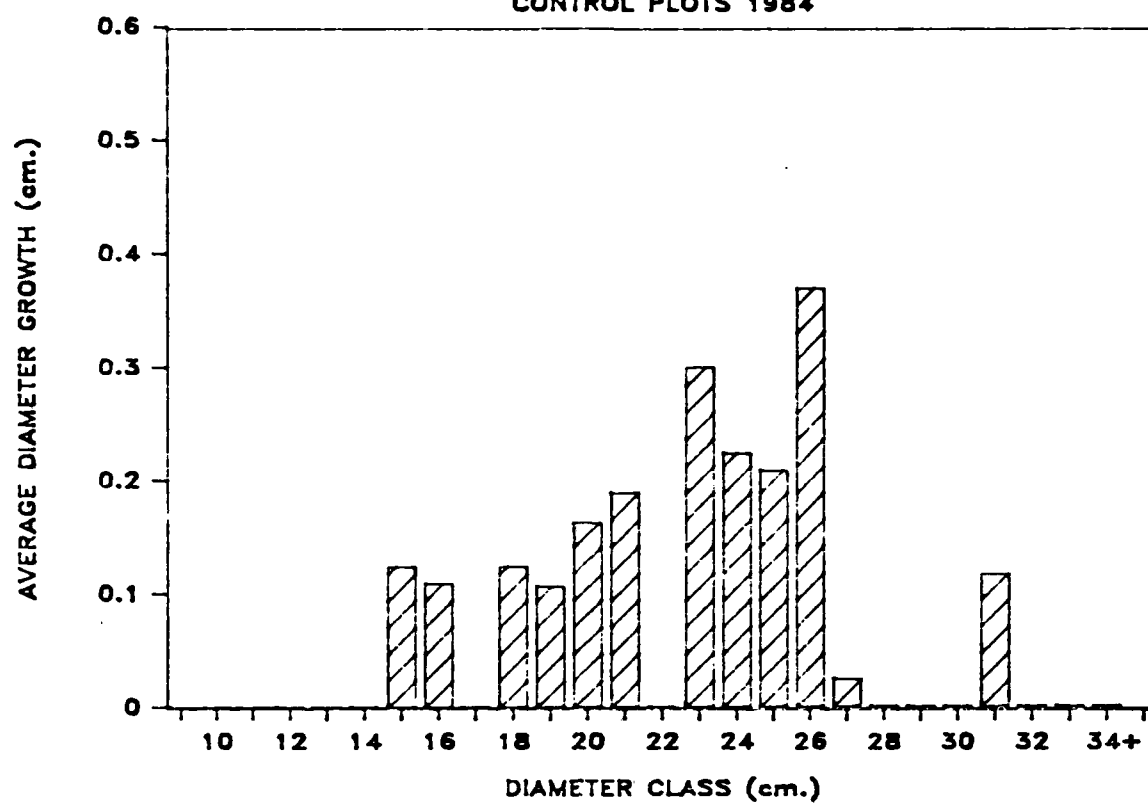
SEASONAL GROWTH — BIG TOOTH ASPEN

ANTENNA PLOTS 1984



SEASONAL GROWTH — QUAKING ASPEN

CONTROL PLOTS 1984



AVERAGE SEASONAL GROWTH BY 1 CM. DIAMETER CLASSES
GROWTH IN CM. OF DIAMETER
ANTENNA - 1984

DBH	NRO	PB	BTA	RM	AVERAGE
10	.000	.000	.000	.079	.079
11	.163	.000	.000	.044	.050
12	.275	.000	.000	.104	.126
13	.180	.201	.521	.061	.126
14	.003	.208	.100	.168	.143
15	.419	.000	.000	.148	.193
16	.000	.000	.000	.174	.174
17	.225	.000	.000	.165	.169
18	.078	.000	.000	.229	.185
19	.134	.024	.000	.154	.111
20	.278	.140	.270	.248	.254
21	.000	.000	.000	.203	.203
22	.264	.000	.437	.112	.242
23	.150	.000	.000	.000	.150
24	.256	.000	.460	.000	.358
25	.117	.000	.567	.132	.272
26	.000	.000	.453	.000	.452
27	.134	.000	.000	.224	.164
28	.000	.268	.407	.000	.337
29	.330	.240	.391	.000	.320
30	.391	.000	.432	.000	.412
31	.292	.000	.000	.000	.292
32	.349	.000	.000	.000	.349
33	.000	.000	.529	.000	.529
34+	.417	.000	.000	.000	.417
<hr/>					
AVERAGE	.247	.172	.428	.140	.185

NRO - NORTHERN RED OAK
PB - PAPER BIRCH
BTA - BIG TOOTH ASPEN
RM - RED MAPLE

AVERAGE SEASONAL GROWTH BY 1 CM. DIAMETER CLASSES
GROWTH IN CM. OF DIAMETER
CONTROL - 1984

DBH	NRO	PB	BTA	RM	QA	AVERAGE
10	.073	.121	.000	.137	.000	.109
11	.008	.039	.000	.204	.000	.123
12	.066	.078	.000	.203	.000	.114
13	.041	.099	.000	.109	.000	.063
14	.079	.003	.000	.000	.000	.064
15	.061	.070	.000	.000	.125	.068
16	.066	.083	.008	.000	.109	.069
17	.102	.079	.159	.000	.000	.104
18	.092	.125	.125	.000	.125	.100
19	.130	.147	.170	.000	.107	.134
20	.132	.124	.234	.000	.163	.149
21	.135	.000	.269	.000	.189	.160
22	.174	.000	.315	.000	.000	.185
23	.179	.102	.361	.000	.300	.218
24	.171	.117	.300	.000	.224	.183
25	.197	.104	.357	.000	.208	.208
26	.160	.000	.353	.000	.368	.209
27	.214	.000	.330	.000	.024	.208
28	.288	.000	.361	.000	.000	.332
29	.000	.000	.000	.000	.000	.000
30	.262	.000	.000	.000	.000	.262
31	.216	.000	.000	.000	.117	.167
32	.235	.000	.000	.000	.000	.235
33	.208	.000	.000	.000	.000	.208
34+	.211	.000	.000	.000	.000	.211
<hr/>						
AVERAGE	.132	.083	.273	.184	.171	.144

NRO - NORTHERN RED OAK
PB - PAPER BIRCH
BTA - BIG TOOTH ASPEN
RM - RED MAPLE
QA - QUAKING ASPEN

NUMBER OF STEMS BY 1 CM. DIAMETER CLASSES
ANTENNA - 1984

DBH	NRO	PB	BTA	RM	TOTAL
10	0	0	0	7	7
11	1	0	0	18	19
12	2	0	0	13	15
13	2	1	1	9	13
14	3	1	1	16	21
15	3	0	0	15	18
16	0	0	0	14	14
17	4	1	0	17	22
18	2	0	0	5	7
19	2	2	0	3	7
20	5	1	1	4	11
21	0	0	0	4	4
22	3	0	1	2	6
23	3	0	0	0	3
24	3	0	3	0	6
25	1	0	1	1	3
26	0	0	2	0	2
27	2	0	0	1	3
28	0	1	1	0	2
29	1	1	1	0	3
30	2	0	2	0	4
31	1	0	0	0	1
32	2	0	0	0	2
33	0	0	1	0	1
34+	3	0	0	0	3
TOTAL	45	8	15	129	197

NRO - NORTHERN RED OAK
PB - PAPER BIRCH
BTA - BIG TOOTH ASPEN
RM - RED MAPLE

APPENDIX I

Timber Volume figures
for research plots.

STATE OF MICHIGAN



NATURAL RESOURCES COMMISSION

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RAYMOND POUPORE

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JAMES J. BLANCHARD, Governor

DEPARTMENT OF NATURAL RESOURCES

RONALD O. SKOOG, Director

Regional Office

1990 South US-41

Marquette, Michigan 49855

January 3, 1985

Mr. Peter Catelino
Michigan Tech. University
Forestry Department
Houghton, MI 49931

Dear Pete:

Here is a breakdown of my cruise of the ELF research plots: (cords)

	<u>Main Line Test</u>	<u>Ground Test</u>	<u>Control</u>
Aspen pulp	32	50	20
Hardwood pulp	17	33	63
Oak sawlogs	1	2	7
Pine sawlogs	<u>6</u>	<u>2</u>	<u>1</u>
	56	87	91

My cruise did not include the 100' wide ROW, the wood less than 4" top d.i.b. or top wood of logs that was used as pulp or firewood.

Sincerely,

Jack Maurer

Project ELF Field Coordinator

Phone: (906)228-6561

JCM:jlk

APPENDIX J

Average values for seedling measurements by
site and date - first growing season

APPENDIX J. Average Values for Seedling Measurements
by Site and Date - First Growing Season

DATE	SITE	SEEDLING CONDITION	BASAL DIAMETER (cm)	PLANT MOISTURE STRESS (bars)	NUMBERS OF MYCORRHIZAL ROOT TIPS	SHOOT ROOT RATIO (WEIGHT BASIS)
JUNE 20 (PLANTING)	GND	-	-	1.2	451	5.59
	ANT	-	-	1.3	348	5.35
	CTL	-	-	1.1	294	5.30
JULY 12	GND	-	-	4.9	-	5.14
	ANT	-	-	3.7	-	5.22
	CTL	-	-	6.8	-	5.21
JULY 24	GND	1.5	0.383	7.7	177	5.82
	ANT	1.3	0.369	8.1	195	6.23
	CTL	1.2	0.366	6.0	207	5.44
AUG 7	GND	1.4	0.360	8.7	-	4.99
	ANT	1.3	0.367	7.8	-	4.01
	CTL	1.4	0.347	7.0	-	5.11
AUG 21	GND	1.6	0.410	13.1	166	5.61
	ANT	1.4	0.388	7.9	159	4.90
	CTL	1.8	0.375	11.8	100	5.67
SEPT 6	GND	2.0	0.376	15.4	-	3.67
	ANT	1.8	0.357	13.6	-	3.81
	CTL	1.5	0.387	11.1	-	3.53
SEPT 20	GND	1.8	0.468	16.5	190	3.95
	ANT	1.5	0.409	16.3	261	4.29
	CTL	1.5	0.449	9.6	182	4.25
OCT 24	GND	1.6	0.465	18.8	289	3.43
	ANT	1.6	0.428	21.0	383	3.97
	CTL	1.4	0.501	11.3	412	4.19

APPENDIX K

Structure of SIR DBMS and description of record
associated with the Trees and Herbaceous Plants Task

STRUCTURE OF SIR DBMS
TREES AND HERBACEOUS PLANTS TASK

PLOT

AMBIENT MONITORING	DENDROMETER BAND	POLE-SIZED TREE MEASUREMENTS	RED PINE SEEDLING MEASUREMENTS
100% TREE INVENTORY	RED PINE MOISTURE STRESS	RED PINE PHENOLOGY	HERBACEOUS BIOMASS EQUATION
MYCORRHIZAL FUNGI COLLECTION	HERBACEOUS PHENOLOGY	HERBACEOUS BIOMASS	HERBACEOUS PERCENT COVER
	MYCORRHIZA CLASSIFICATION FOUND ON ROOT GROWTH	LITTER TRAP DATA	FOLIAGE SAMPLE DATA

Description of records associated with the Trees and Herbaceous Plants Tasks. Each record is keyed to plot number.

Ambient Monitoring Data:

Element: 2

Contents: Daily precipitation, air temperature, relative humidity, soil temperature and soil moisture values.

Collected on: All plots

Collection cycle: Daily

Dendrometer Band Data:

Element: 3

Contents: Diameter measurements of all trees 10 cm and larger.

Collected on: Control and antenna pole-sized tree plots.

Collection Cycle: Weekly during the growing season.

Pole-sized Tree Measurement Data:

Element: 3

Contents: Yearly observation of tree height, diameter, crown position and tree condition on all trees 10 cm and larger.

Collected on: Control and antenna pole-sized tree plots.

Collection Cycle: Yearly

Red Pine Seedling Measurement Data:

Element: 3

Contents: Yearly observation of seedling height, diameter, condition and bud set on 100 red pine seedlings/plot.

Collected on: Control, ground and antenna plantation plots.

Collection Cycle: Yearly

Red Pine Moisture Stress Data:

Element: 3

Contents: Seedling height, diameter, condition, plant moisture stress value, top and root weight and mycorrhizal counts.

Collected on: Control, ground and antenna plantation plots.

Collection Cycle: Biweekly during growing season.

100% Tree Inventory:

Element: 3

Contents: Tree measurements on all live and dead trees 2.5 cm and larger.

Collected on: Control and antenna pole-sized tree plots.

Collection Cycle: Once

Red Pine Phenology:

Element: 4

Contents: Red pine seedling diameter, height, bud burst and candle elongation values.

Collected on: Control, ground and antenna plantation plots.

Collection Cycle: Weekly during the growing season.

Herbaceous Percent Cover:

Element: 5

Contents: Herbaceous plant coverage measurements.

Collected on: Control and antenna site herbaceous reserves and plantation.

Collection Cycle: Yearly

181

Herbaceous Biomass:

Element: 5

Contents: Herbaceous biomass measurements.

Collected on: Control and pole-sized tree plots.

Collection Cycle: Once

Herbaceous Biomass Equation Data:

Element: 5

Contents: Herbaceous biomass measurements used in the development of herbaceous biomass equations.

Collected on: Control and antenna tree pole-sized plots.

Collection Cycle: Once

Mycorrhizal Fungi Collection:

Element: 6

Contents: Counts of fruiting bodies by isolated mycorrhizal fungi species/categories.

Collected on: All plots.

Collection Cycle: 10-18 times/year.

Mycorrhizal Classification:

Element: 7

Contents: Population counts of isolated mycorrhizal fungi species/categories.

Collected on: All plots.

Collection Cycle: Twice/year - early spring and late fall.

Litter Trap Data:

Element: 8

Contents: Nutrient and weight values of litter components.

Collected on: Control and antenna pole-size tree plots.

Collection Cycle: Variable - monthly to weekly during the growing season.

Foliage Sample Data:

Element: 8

Contents: Nutrient values of leaves.

Collected on: Vicinity of pole-sized tree plots at the antenna and control sites.

Collection Cycle: monthly

ELF COMMUNICATIONS SYSTEM ENVIRONMENTAL MONITORING PROGRAM:
LITTER DECOMPOSITION AND MICROFLORA
The Michigan Study Site

ANNUAL REPORT, 1984

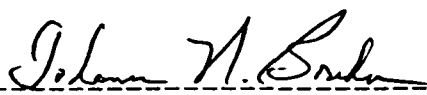
SUBCONTRACT NUMBER: E06549-84-C-002

MICHIGAN TECHNOLOGICAL UNIVERSITY
HOUGHTON, MICHIGAN

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The Michigan Study Site

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TABLE OF CONTENTS

SUMMARY -----	1
INTRODUCTION -----	3
ELEMENT 1. LITTER DECOMPOSITION AND NUTRIENT FLUX -----	5
Introduction -----	5
Methods -----	5
Description of Progress -----	9
ELEMENT 2. RHIZOPLANE AND RHIZOSPHERE ACTINOMYCETES -----	53
Introduction -----	53
Methods -----	54
Description of Progress -----	58
LITERATURE CITED -----	68
APPENDIX A. DESCRIPTION OF STREPTOMYCETE TYPES -----	71

SUMMARY

The 1984 field season represents the first full year of study at the ELF antenna and ground sites. Final selection of a control site was made too late to be included in the 1983-84 litter decomposition/ nutrient flux experiment. However, the control site is included in the 1984-85 study. Plantation sites were cleared in June. Litter decomposition/ nutrient flux sample materials were shifted off of the Antenna and Ground site plantation locations for one week during the clearing and planting process and then replaced on the freshly cleared plantations. Study of streptomycetes associated with red pine seedling mycorrhizae commenced with planting. The feasibility of studying streptomycete populations associated with decomposing litter samples was also investigated.

Work in 1984 concentrated on red pine as the sole study tree species. However, the 1984-85 litter decomposition/ nutrient flux experiment, established in early December, 1984, was broadened to include monthly samples of northern red oak and red maple as well as red pine. The 1984-85 study includes all three plantations and the two pole stands. Rhizosphere streptomycete work will continue to focus on the three red pine plantations, and may be broadened to consider foliar litter samples retrieved from the pole-stands and/or plantations. Both work elements will make use of the ambient monitoring system operated and maintained by the Herbaceous Plant Cover and Tree Studies project (Element 2), and both work elements will remain closely tied to the litter production (Element 8) and mycorrhiza (Elements 6 and 7) study elements of that project.

Data from the 1983-84 study show that the patterns of overall and nutrient mass loss between locations were very similar. No significant differences were detected for overall mass loss or for change in total nitrogen content between locations for given dates. However, litter placed in the Antenna site pole-stand lost phosphorus, potassium, calcium and magnesium more slowly than did litter placed in the plantations, or even

recovered nutrient mass (especially potassium) late in the season.

Emphasis in 1985 in the decomposition/ nutrient flux element will focus on adding northern red oak and red maple to red pine as test species. The influence of litter fragmentation on apparent litter decomposition rates will be determined.

Emphasis in 1985 in the rhizoplane actinomycete element will be placed on increasing replication. This will be accomplished by eliminating soil samples and heterotrophic bacteria (other than streptomycetes) from the test matrix, and by concentrating on use of the enrichment technique developed for isolation of streptomycetes from mycorrhizae and litter samples. Nursery samples were included initially to characterize the original mycorrhizal and bacterial populations on the root systems of the red pine planting stock used in this study. No further nursery sampling will be conducted.

INTRODUCTION

The litter decomposition subsystem of any forest ecosystem serves to 1) transform the essential plant nutrients in organic matter into forms available for root uptake, 2) pool the nutrients collected by primary producers, and 3) release these nutrients in a regulated fashion for re-use by the autotrophs. The energy provided by litter decomposition also fuels heterotrophic dinitrogen fixation and capture of nutrients washed from the atmosphere or leached from living plants. Due to the large quantities of potentially available plant nutrients found in the litter component of forest biomass, knowledge of key decomposition processes and their rates is essential to conceptualization of ecosystem dynamics. Organic matter decomposition is primarily accomplished by heterotrophic microorganisms whose activities are regulated by the environment. Recognizing the delicate balance of ecosystem functioning, it is apparent that environmental factors which disrupt decomposition processes detract from the optimum flow of nutrients to vegetation. As one such environmental factor, ELF electromagnetic fields merit investigation for possible effects on the litter decomposition subsystem.

Litter decomposition is a complex process involving a variety of organisms engaged in the degradation of a wide range of organic compounds. The primary agents of organic matter decomposition are the fungi and bacteria. Within these broad groups, a relatively small cadre are responsible for degradation of complex structural materials such as cellulose and lignin. Among the fungi, cellulose and lignin degradation are accomplished by members of the Basidiomycetes and Ascomycetes. Of the bacteria, members of the Actinomycetes have been found to degrade cellulose and lignin/lignocellulose in both coniferous and deciduous litter systems.

The broad objectives of this study are: 1) to characterize a) the rates of foliar organic matter decomposition and nutrient cycling and b) populations of mycorrhiza-associated

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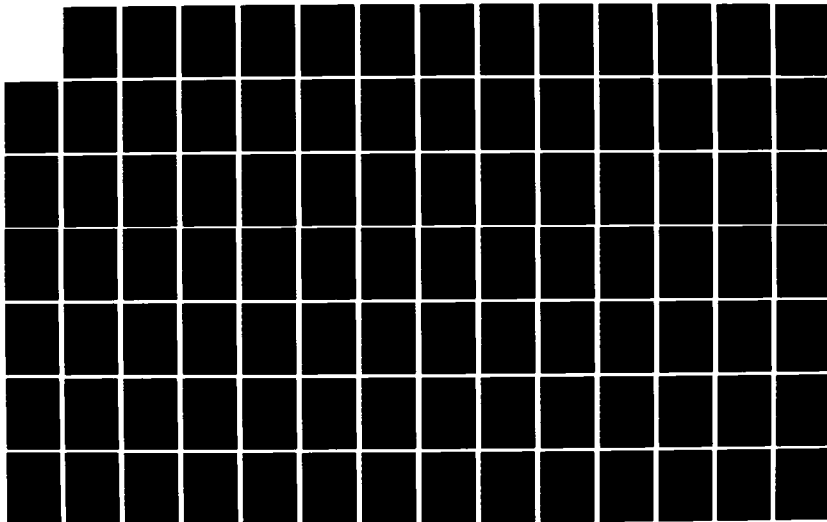
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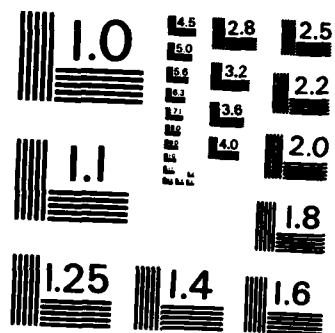
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streptomycetes on selected sites within the ELF antenna area prior to operation of the antenna, and 2) to use these baseline data to evaluate possible ELF field effects on these sensitive processes and populations. Although the two work elements represented in this project (reflected in 1a and 2b above) may appear disjointed, the relevance of each element is readily apparent when viewed in conjunction with the Herbaceous Plant Cover and Tree Species project as an integrated whole.

Element 1: Litter Decomposition and Nutrient Flux

Introduction

Overall litter mass loss has traditionally been used as a measure of fully integrated litter decomposition. It has been shown, however, that both the accuracy and precision of mass loss as a sensitive index of organic matter deterioration declines with time beyond approximately one year, while nutrient flux provides continuously meaningful ecological information. Microfloral population shifts have been shown to influence the rate of overall litter decomposition (Mitchell and Miller 1978). Conversely, overall litter mass loss and nutrient flux are useful measures of the impact of environmental perturbations on the integrated activities of the litter biota. ELF fields represent one possible cause of perturbations.

Litter decomposition/ nutrient flux studies greatly extend the usefulness of litter productivity data collected in the course of forest vegetation studies. Knowledge of litter biomass production and nutrient content likewise serves as the basis for decomposition study. Further, the study methods employed integrate the activities of the microflora with all but the largest arthropods and earthworms, extending the value of all population data.

Since the 1983 Annual Report was written, an entire year's experience with red pine foliar litter decomposition and nutrient flux has been gained on the Ground and Antenna sites. Experience gained supports the contention that overall and nutrient mass loss over time from freshly fallen foliar litter can be characterized with sufficient precision to detect environmental perturbation

Methods

Litter decomposition is being quantified as percent change over time in overall mass and nutrient (N, P, K, Ca, Mg, and S) masses. Analysis of litter nutrient content is being conducted by the Soils Analysis Laboratory, Department of Forestry,

Michigan Technological University.

1983-84 Study.-- Fresh-fallen red pine litter was collected from the LaCroix red pine plantation near Houghton due to 1) its proximity to MTU, and 2) its relative remoteness from interfering electromagnetic fields. A single parent collection at one location avoids differences which might be present in substrate quality between different stands on different sites.

Red pine overall mass loss was studied by the tethered fascicle method, as well as via bulk litter samples, while nutrient flux was studied solely with bulk litter samples. Fascicles offer the opportunity to study litter decomposition without the errors due to litter losses from and inputs to envelopes over the course of an experiment. Fascicles broken during the course of an experiment can be discarded prior to analysis.

Fresh:dry mass ratios and initial nutrient content were determined for 10 random samples taken from the LaCroix plantation parent collection. All mass loss data (overall as well as nutrient masses) are based on 30°C dry masses. Random subsamples from the parent litter collection were placed in replicate nylon mesh envelopes (3 mm nylon mesh). Four bulk litter envelopes measuring 22 cm x 28 cm, each containing 10 g (air dry weight) of the parent collection and two envelopes measuring 22 cm x 14 cm, each containing 10 perfect preweighed and tethered pine needle fascicles, were disbursed at five random locations on each of the nine study plots (three plots each at Antenna site pole-stand and plantation and at the Ground plantation). No control site was included because our tentative control site was disqualified by IITRI late in the field season and an alternate site remained to be established. The 1983-84 decomposition experiment is restricted to red pine due to the relatively low levels of precision attainable in characterizing paper birch litter decomposition/nutrient flux.

Sample sizes were based on the results of the 1982-83 pilot study at Martels Lake, and on the intent to retrieve samples only twice, once in the early spring and one year after disbursal. It

was ultimately decided, though, to retrieve samples on a monthly basis during 1984, in order to follow more closely the progress of decomposition and nutrient flux. Sufficient samples existed to permit monthly sampling of both bulk and fascicle samples from early May through early December for comparison of the three sites, if not the nine plots. Snow cover at the study sites dictates the earliest recovery date, as samples are frozen to the ground until snowmelt is complete. Sufficient samples were recovered each month to permit both 1) analysis of differences in overall and nutrient masses between dates and sites by t-tests and 2) attempts to fit single and double exponential models to plots of overall mass loss against time for statistical comparison of rate constants between sites (Wieder and Lang 1982). Lack of ambient monitoring data during most of 1984 prevented evaluation of the influence of environmental variables on decomposition through analysis of covariance.

In order to further compare the bulk litter and tethered fascicle methods of quantifying decomposition, moisture content in situ was determined for each sample at the time of retrieval. Each retrieved sample was placed in an air-tight plastic freezer-storage bag from which as much air as possible was then removed. Fresh wet weights were recorded in the laboratory prior to drying to a constant weight at 30°C, which was also recorded. Moisture content at the time of retrieval was calculated as wet weight minus dry weight divided by dry weight. The influence of moisture on decomposition via the bulk sample versus the tethered fascicle method will be evaluated through analysis of covariance.

1984-85 Study.-- Fresh-fallen red pine litter was again collected on polyethylene tarps spread in the LaCroix red pine plantation near Houghton. Fresh-fallen red maple litter was collected along the Covered Drive, seven miles from Houghton, again due to 1) its proximity to MTU, and 2) its relative remoteness from interfering electromagnetic fields. Northern red oak litter was collected adjacent to the Control site plantation.

Samples of foliar litter representing all three species will be retrieved monthly from 15 plots (3 plots each in Ground,

Antenna and Control site plantations, and in Antenna and Control site pole-stands). Nutrient mass loss from foliar litter of all three species will be determined from bagged bulk litter samples. In addition to overall mass loss estimates from bulk samples, overall mass loss will be determined for bagged individual tethered fascicles and leaves. Each tethered fascicle or leaf was perfectly intact at the time of disbursal, so that fragmentation associated with decomposition can be quantified. Finally, in order to compare bagged with unbagged specimens, tethered, unbagged individual pine fascicles and oak leaves were also placed in the field on one plot each of the plantation and pole-stand at the Control site.

Ambient monitoring data and litter moisture content at the time of retrieval will serve as covariates in analysis of covariance to factor routine environmental variables out of comparisons of decomposition progress between sites, dates and years. Ambient monitoring variables which will receive special attention include air temperature and precipitation, as well as soil moisture and temperature at 5 cm depth.

The relative advantages and disadvantages of basing statistical tests on median values rather than on mean values will be explored. Median-based statistics might be useful because relatively small sample sizes are involved. Each month, from 1 April through 1 December, 1985, two bulk sample envelopes and one tethered specimen envelope (containing 10 fascicles or leaves) for each species will be retrieved from each plot. Also, one set of 10 tethered unbagged pine fascicles and oak leaves will be retrieved from the Control site plantation and pole-stand. As a result, decomposition estimates will be based on 6 bulk samples and as many as 30 fascicles or leaves (depending on fragmentation) for each species on each site and date. In addition to analysis of mass loss data, sufficient samples will be collected frequently enough to permit the fitting of exponential models to overall mass loss data collected at each site using the program BMDPAR. The overall form of the resulting models can be compared statistically using t-tests for

differences in the calculated rate constants.

Fragmentation will be studied by determining the per cent of leaf surface area lost over time by individual oak and maple leaves. Photocopy replicas of the individual tethered leaves placed in the field will be used for comparison of initial and final leaf dimensions. Leaf surface areas will be determined using a photoelectric leaf area meter. It may be possible to weigh overall mass loss data for oak and maple by a factor which accounts for fragmentation. The 1985-86 study may include an experiment to estimate the rate of decomposition of litter fragments which filter into the fermentation layer of the forest floor. Such fragments should decompose faster than the litter remaining at the forest floor surface (Witkamp and Olson 1963). Characterization of these accelerated rates would enhance the potential to accurately model first year decomposition processes at the study sites.

Description of Progress

1983-84 Study.-- Tables 1 through 7 present changes in overall mass (for both bulk samples and individual fascicle samples) and nutrient masses which took place during the first year of red pine foliage decomposition at three study locations. Preliminary statistical analyses have been conducted by using the t-test for unpaired data to compare means for the three study locations within individual recovery dates ($\alpha=0.05$). Figures 1 through 28 present the same data graphically. The similarity in overall mass loss progress over time at the three study locations is borne out by the single exponential models derived from the data collected at each location. Three variations of the single exponential model were evaluated (Table 8, Figures 29-34). The simplest form does not include a lag period (Figures 29 and 30). Because litter decomposition occurred over the winter months, the residual sums of squares for all three locations were large. The second and third forms of single exponential model both incorporated a lag period. The second form (Figures 31 and 32) selected independent lag factors for each location and method,

Table 1. Mean proportions^a of initial overall mass (30°C) remaining at different times in 1984, for bulk red pine foliar litter samples disbursed in early December, 1983.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	0.92 (0.01) ^b	0.91 (0.01)	0.91 (0.01)
1 June	0.92 (0.01)	0.92 (0.01)	0.91 (0.01)
1 July	0.89 (0.02)	0.90 (0.00)	0.89 (0.01)
1 August	0.88 (0.00)	0.88 (0.01)	0.87 (0.00)
1 September	0.83 (0.01)	0.82 (0.01)	0.81 (0.02)
1 October	0.79 (0.02)	0.80 (0.02)	0.78 (0.01)
1 November	0.73 (0.05)	0.75 (0.02)	0.76 (0.02)
1 December	0.75 (0.01)	0.75 (0.03)	0.75 (0.02)

a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 represent the 30°C dry weights of samples initially and at time 1, respectively. Dry weight at time 0 was estimated from fresh to dry weight (30°C) ratios determined for separate random subsamples taken at the time of litter sample preparation. These samples were also used to determine initial nutrient content.

b/ standard deviation

Table 2. Mean proportions^a of initial overall mass (30°C) remaining at different times in 1984, for individual tethered red pine litter fascicles disbursed in early December, 1983.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	0.90 (0.02) ^b	0.91 (0.01)	0.90 (0.02)
1 June	0.92 (0.02)	0.92 (0.02)	0.91 (0.02)
1 July	0.91 (0.03)	0.90 (0.03)	0.88 (0.03)
1 August	0.87 (0.03)	0.88 (0.02)	0.87 (0.03)
1 September	0.82 (0.04)	0.80 (0.02)	0.81 (0.03)
1 October	0.77 (0.03)	0.78 (0.03)	0.76 (0.02)
1 November	0.74 (0.04)	0.73 (0.02)	0.75 (0.03)
1 December	0.74 (0.03)	0.75 (0.02)	0.74 (0.03)

a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 represent the 30°C dry weights of samples initially and at time 1, respectively. Dry weight at time 0 was estimated from fresh to dry weight (30°C) ratios determined for separate random subsamples taken at the time of litter sample preparation.

b/ standard deviation

Table 3. Mean proportions^a of initial total N content (w/w, 30°C) remaining in bulk red pine foliar litter samples at different times during 1984, following early December, 1983, disbursal.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May ^f	----	----	----
1 June ^f	----	----	----
1 July ^f	0.87	0.93	----
1 August	1.02 (0.07) ^b	1.00 (0.04)	1.06 (0.05)
1 September	0.96 (0.05)	1.16 (0.30)	1.17 (0.17)
1 October	1.10 (0.06)	1.18 (0.09)	1.13 (0.06)
1 November	1.01 (0.12)	1.07 (0.09)	1.13 (0.19)
1 December	1.04 (0.04)	1.05 (0.14)	1.03 (0.06)

- a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 are the percentages of N (w/w, 30°C) multiplied by total dry weight (30°C) for time 0 and time 1, respectively.
b/ standard deviation
c/ Plantations differ significantly ($\alpha = 0.05$).
d/ Ground plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).
e/ Antenna plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).
f/ Data will be available for the final draft of this report.

Table 4. Mean proportions^a of initial total P content (w/w, 30°C) remaining in bulk red pine foliar litter samples at different times during 1984, following early December, 1983, disbursal.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	1.01 (0.04) ^b	1.02 (0.04)	1.03 (0.06)
1 June	0.98 (0.07)	1.02 (0.02)	1.03 (0.06)
1 July	0.92 (0.12)	0.88 (0.09)	0.99 (0.01)
1 August ^d	0.76 (0.05)	0.75 (0.08)	0.86 (0.04)
1 September ^{cde}	0.68 (0.04)	0.62 (0.04)	0.75 (0.05)
1 October ^{de}	0.61 (0.02)	0.63 (0.06)	0.73 (0.04)
1 November ^{de}	0.57 (0.06)	0.58 (0.04)	0.74 (0.05)
1 December ^{de}	0.61 (0.04)	0.59 (0.09)	0.79 (0.04)

- a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 are the percentages of P (w/w, 30°C) multiplied by total dry weight (30°C) for time 0 and time 1, respectively.
b/ standard deviation
c/ Plantations differ significantly ($\alpha = 0.05$).
d/ Ground plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).
e/ Antenna plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

Table 5. Mean proportions^a of initial total K content (w/w, 30°C) remaining in bulk red pine foliar litter samples at different times during 1984, following early December, 1983, disbursal.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	0.14 (0.01) ^b	0.15 (0.01)	0.14 (0.01)
1 June	0.16 (0.02)	0.14 (0.01)	0.13 (0.01)
1 July	0.16 (0.02)	0.18 (0.03)	0.14 (0.01)
1 August ^d	0.10 (0.01)	0.09 (0.01)	0.13 (0.02)
1 September ^{cde}	0.08 (0.02)	0.06 (0.00)	0.13 (0.02)
1 October ^d	0.06 (0.01)	0.06 (0.01)	0.14 (0.02)
1 November ^d	0.07 (0.02)	0.09 (0.03)	0.22 (0.03)
1 December ^d	0.09 (0.02)	0.06 (0.01)	0.20 (0.02)

a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 are the percentages of K (w/w, 30°C) multiplied by total dry weight (30°C) for time 0 and time 1, respectively.

b/ standard deviation

c/ Plantations differ significantly ($\alpha = 0.05$).

d/ Ground plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

e/ Antenna plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

Table 6. Mean proportions^a of initial total Ca content (w/w, 30°C) remaining in bulk red pine foliar litter samples at different times during 1984, following early December, 1983, disbursal.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	1.07 (0.05) ^b	1.07 (0.08)	1.09 (0.10)
1 June	0.97 (0.11)	1.00 (0.03)	1.05 (0.03)
1 July	1.06 (0.17)	1.04 (0.09)	1.15 (0.03)
1 August	0.93 (0.06)	0.81 (0.11)	0.95 (0.06)
1 September	0.89 (0.02)	0.87 (0.05)	0.92 (0.03)
1 October	0.91 (0.12)	0.94 (0.06)	0.99 (0.06)
1 November ^e	0.94 (0.07)	0.88 (0.06)	1.01 (0.04)
1 December ^d	0.81 (0.07)	0.82 (0.04)	0.95 (0.07)

a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 are the percentages of Ca (w/w, 30°C) multiplied by total dry weight (30°C) for time 0 and time 1, respectively.

b/ standard deviation

c/ Plantations differ significantly ($\alpha = 0.05$).

d/ Ground plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

e/ Antenna plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

Table 7. Mean proportions^a of initial total Mg content (w/w, 30°C) remaining in bulk red pine foliar litter samples at different times during 1984, following early December, 1983, disbursal.

Sample Retrieval Date	Ground Plantation	Antenna	
		Plantation	Pole-stand
11 May	0.75 (0.04) ^b	0.80 (0.05)	0.75 (0.03)
1 June	0.75 (0.06)	0.78 (0.05)	0.76 (0.04)
1 July	0.75 (0.05)	0.73 (0.01)	0.71 (0.07)
1 August	0.63 (0.02)	0.55 (0.09)	0.64 (0.03)
1 September ^d	0.50 (0.04)	0.48 (0.03)	0.60 (0.05)
1 October ^d	0.40 (0.04)	0.42 (0.05)	0.52 (0.04)
1 November ^d	0.35 (0.03)	0.36 (0.04)	0.55 (0.10)
1 December ^d	0.36 (0.06)	0.31 (0.04)	0.56 (0.07)

a/ Proportion ($X=W_1/W_0$), where W_0 and W_1 are the percentages of Mg (w/w, 30°C) multiplied by total dry weight (30°C) for time 0 and time 1, respectively.

b/ standard deviation

c/ Plantations differ significantly ($\alpha = 0.05$).

d/ Ground plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

e/ Antenna plantation differs significantly from Antenna pole-stand ($\alpha = 0.05$).

while the third form (Figures 33 and 34) forced a lag period of 89 days (the mean lag period derived for the bulk and individual fascicle equations at the three locations). Lag periods varied in length, from 85 to 95 days following December 1 disbursal. Differences in lag period between locations and between bulk and individual fascicle methods were not significant ($\alpha = 0.05$). Confidence intervals based on t-tests were calculated in order to determine whether or not differences between decomposition rate constants derived for different locations or methods were significant ($\alpha = 0.05$). The only apparently significant difference was between the Antenna site pole-stand and the two plantations, using the individual fascicle data and the model lacking a lag period. Because this model provided the poorest fit to the actual data based on comparison of residual sums of squares, this difference in decomposition rate constants is probably meaningless. It is quite clear that overall pine litter mass loss proceeded at similar rates at both the Antenna and Ground sites, in both plantations and the pole-stand, and as determined by bulk litter samples and individual pine fascicles. Moisture content differed little between bulk samples and

Figures 1 through 8.

Figure 1. Summary of overall mass loss by bulk pine litter samples at all three study locations between 1 December, 1983, and 1 December, 1984.

Figure 2. Overall mass loss by bulk pine litter samples on the Ground site plantation between 1 December, 1983, and 1 December, 1984.

Figure 3. Overall mass loss by bulk pine litter samples on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.

Figure 4. Overall mass loss by bulk pine litter samples in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.

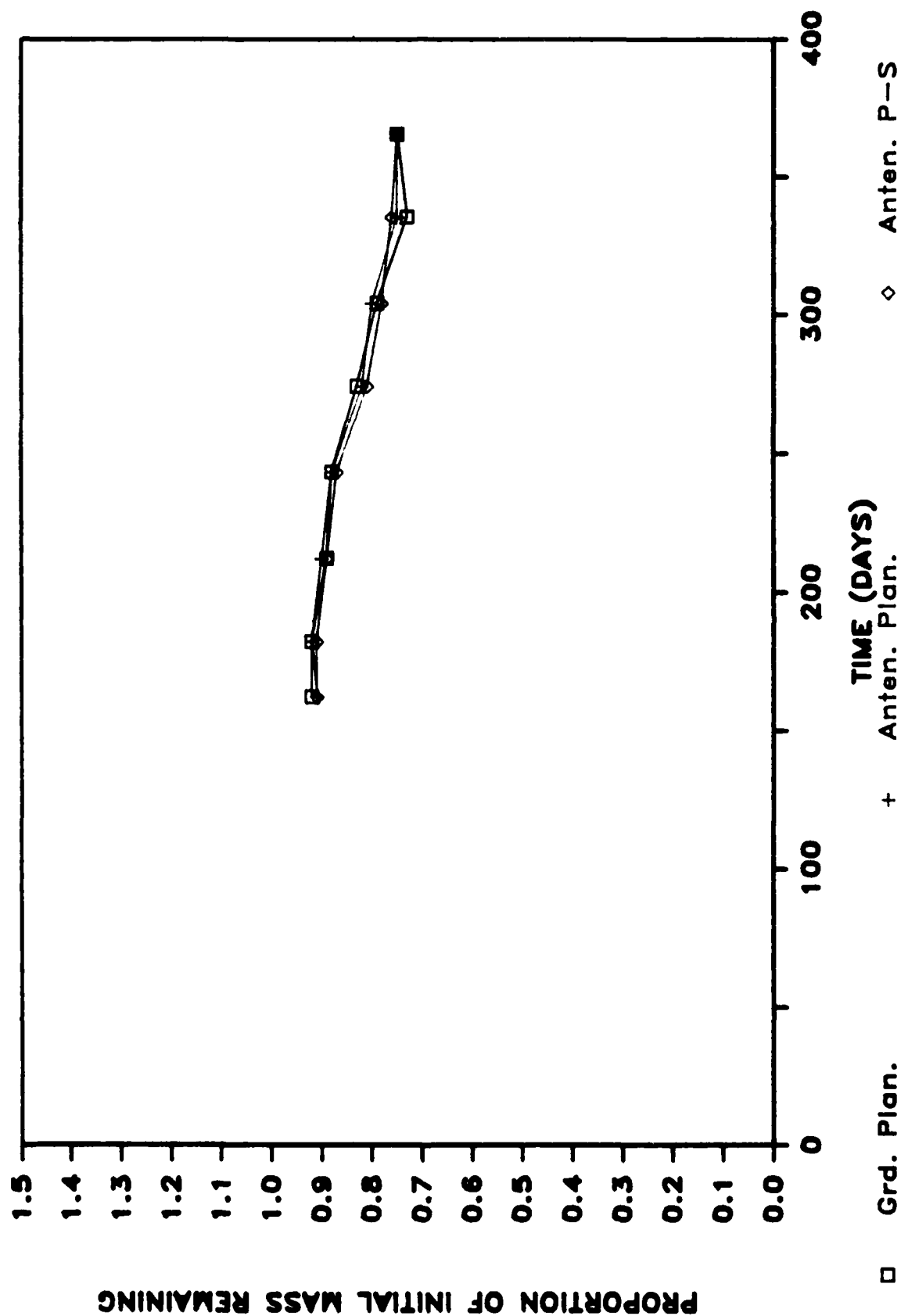
Figure 5. Summary of overall mass loss by individual pine fascicles at all three study locations between 1 December, 1983, and 1 December, 1984.

Figure 6. Overall mass loss by individual pine fascicles on the Ground site plantation between 1 December, 1983, and 1 December, 1984.

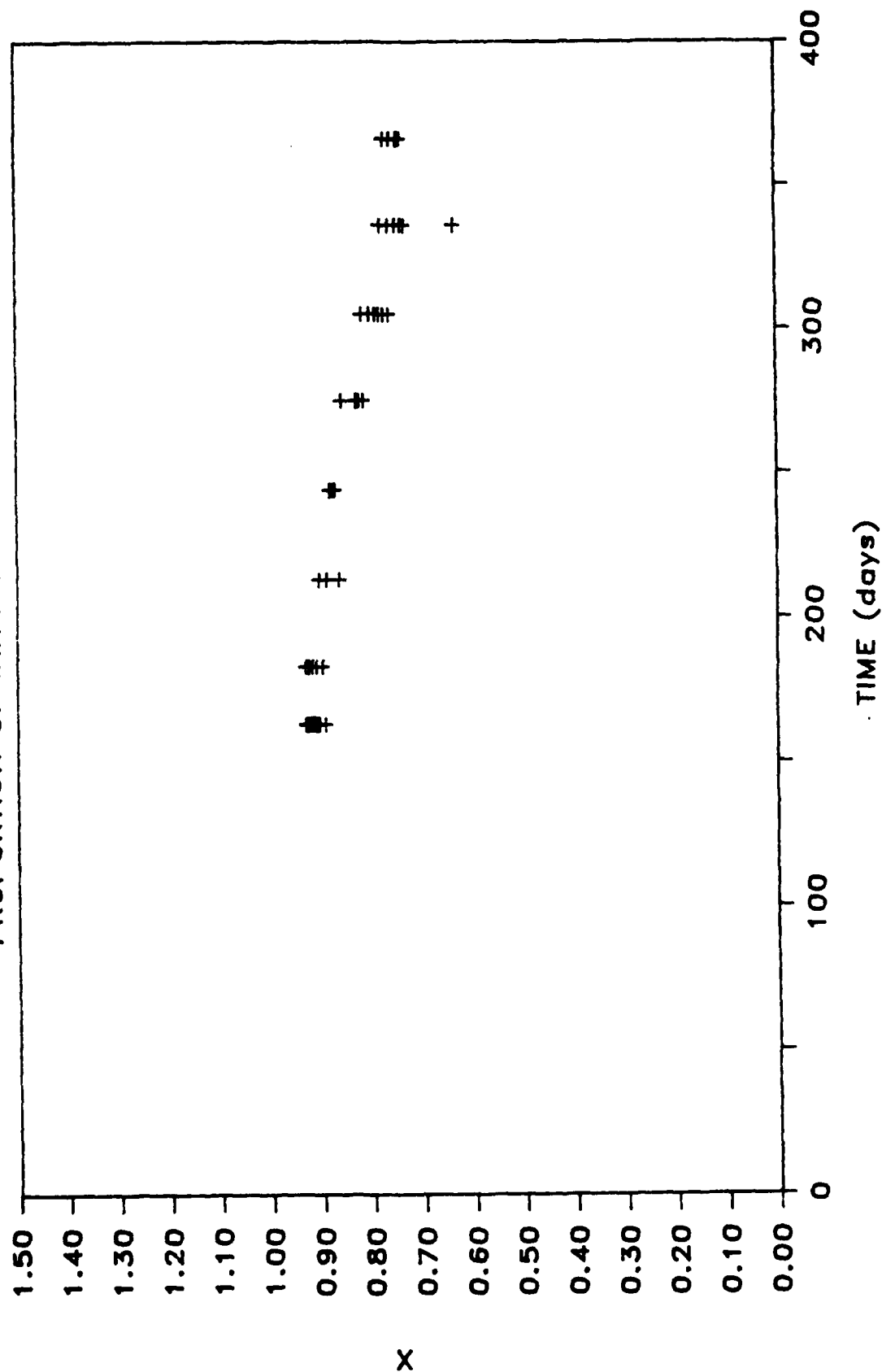
Figure 7. Overall mass loss by individual pine fascicles on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.

Figure 8. Overall mass loss by individual pine fascicles in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.

TOTAL MASS, BULK LITTER ENVELOPES

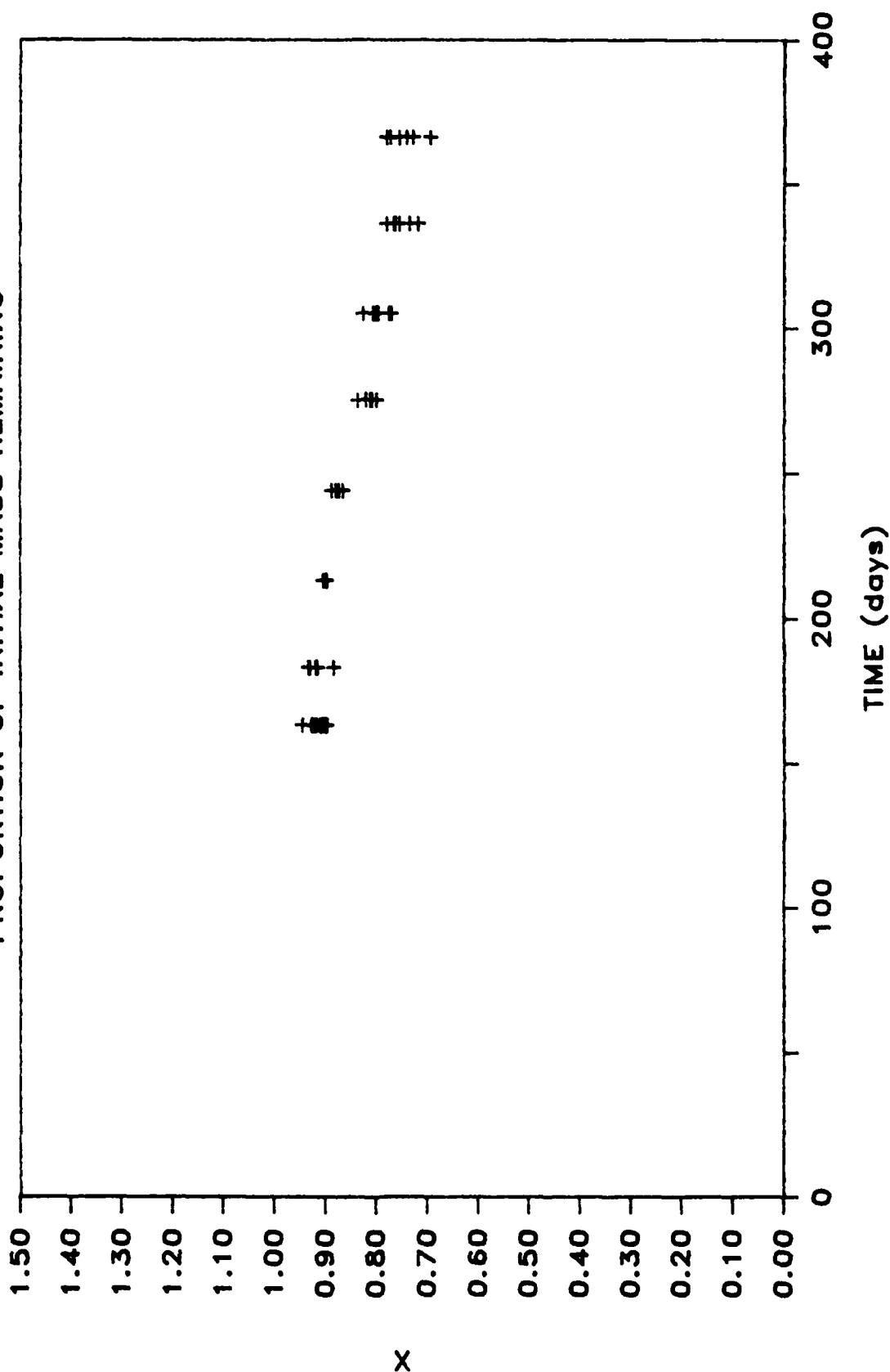


BULK PINE LITTER, GROUND PLANTATION



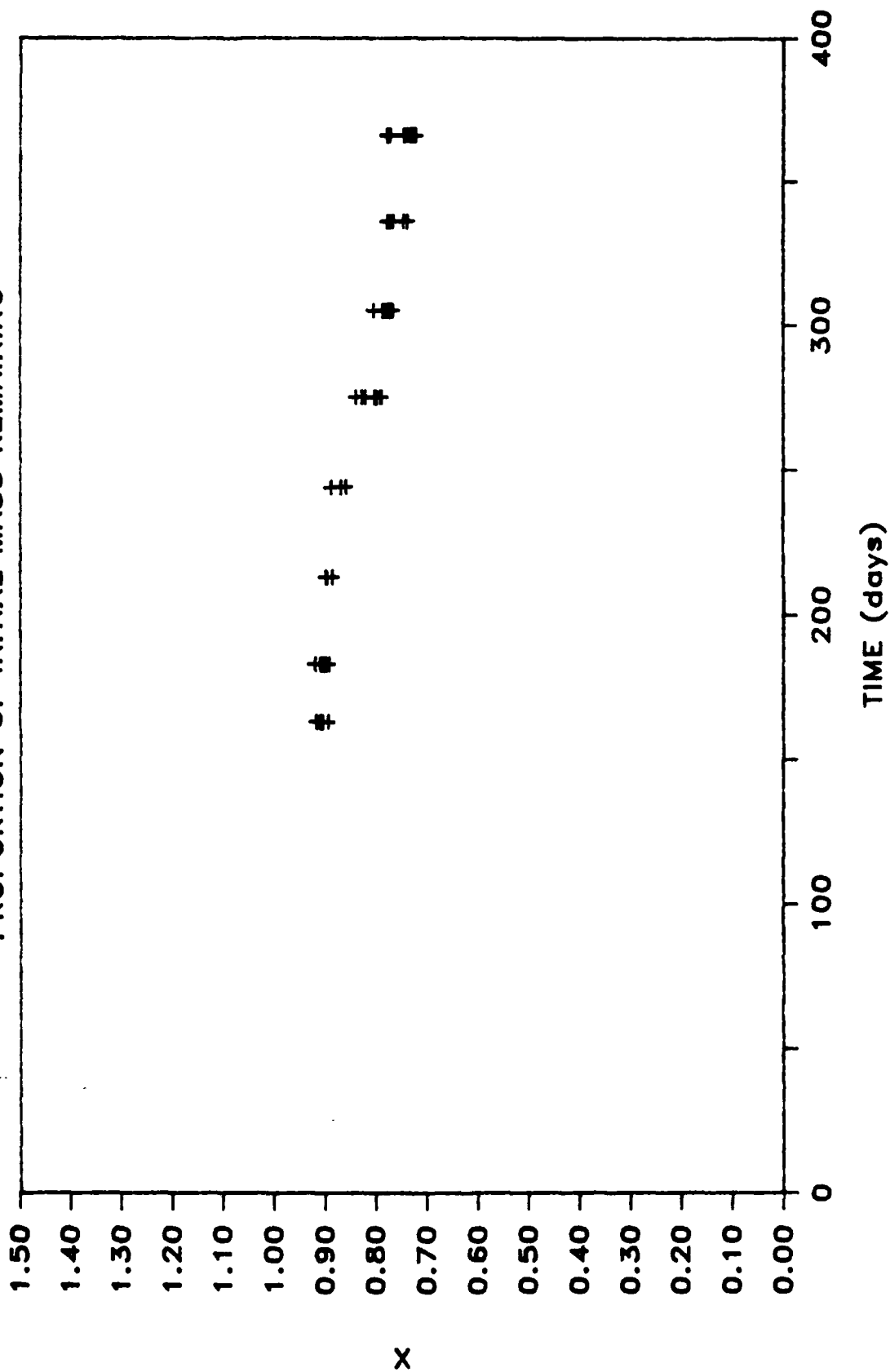
BULK PINE LITTER, ANTENNA PLANTATION

PROPORTION OF INITIAL MASS REMAINING

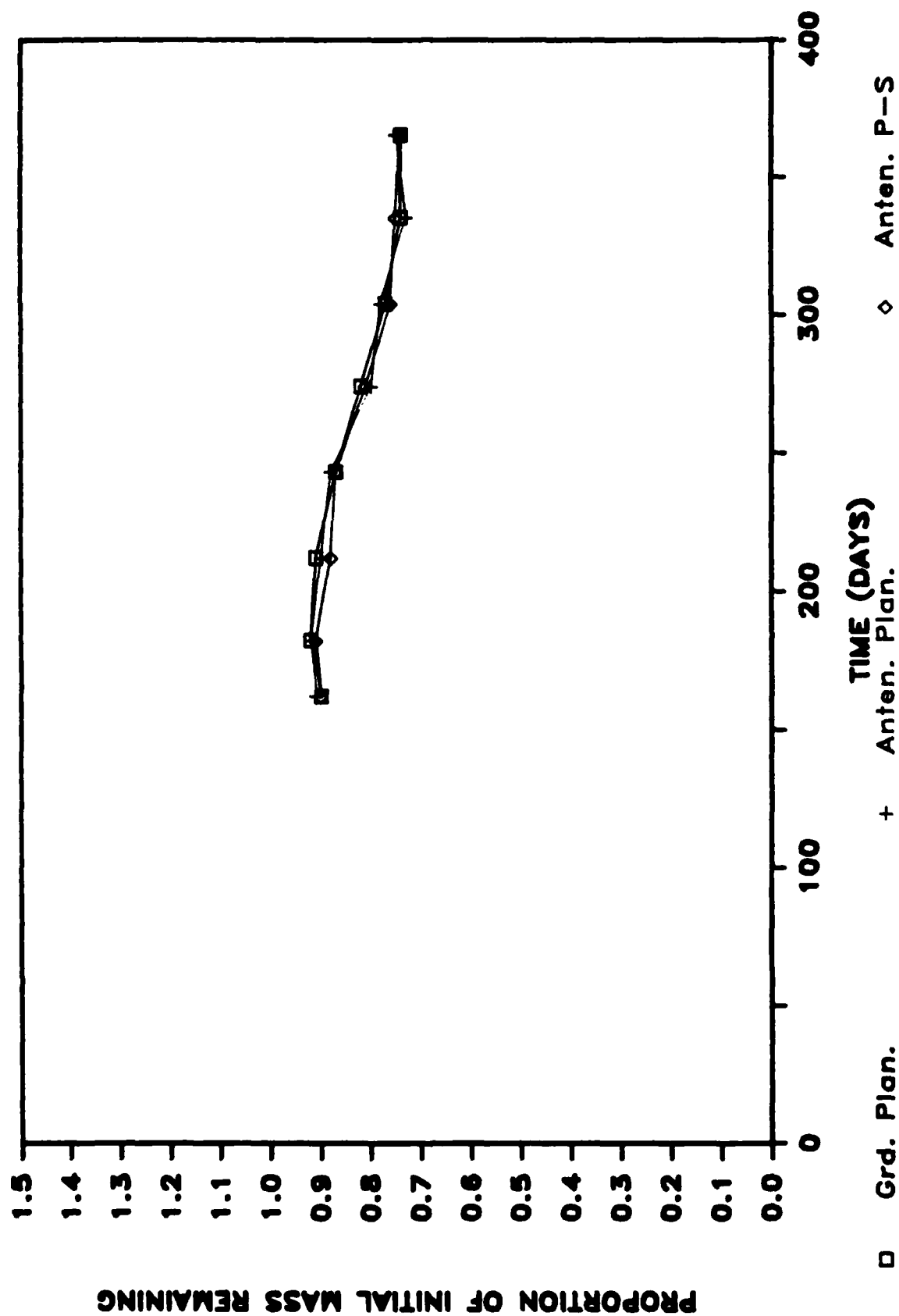


BULK PINE LITTER, ANTENNA POLE-STAND

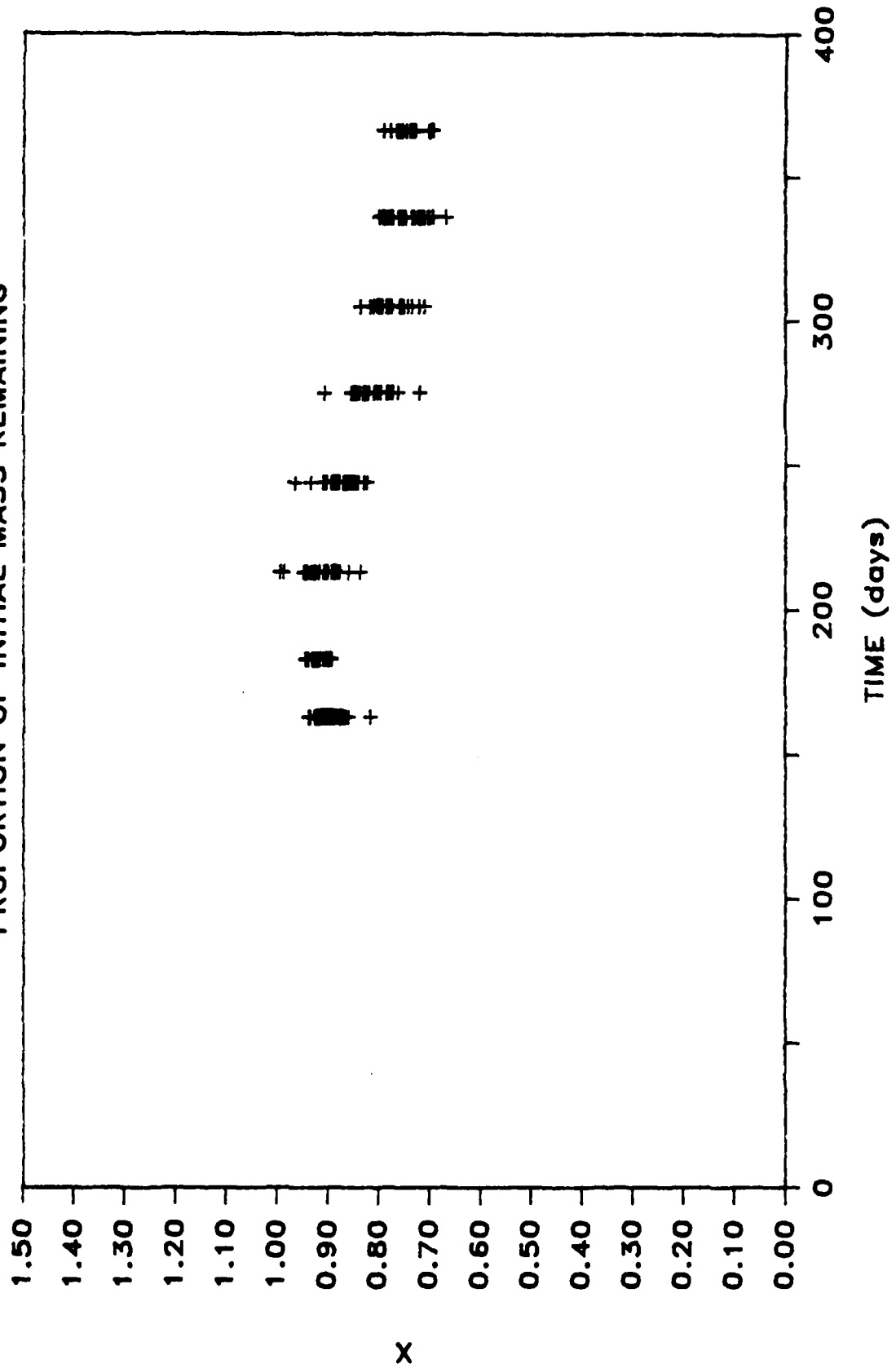
PROPORTION OF INITIAL MASS REMAINING



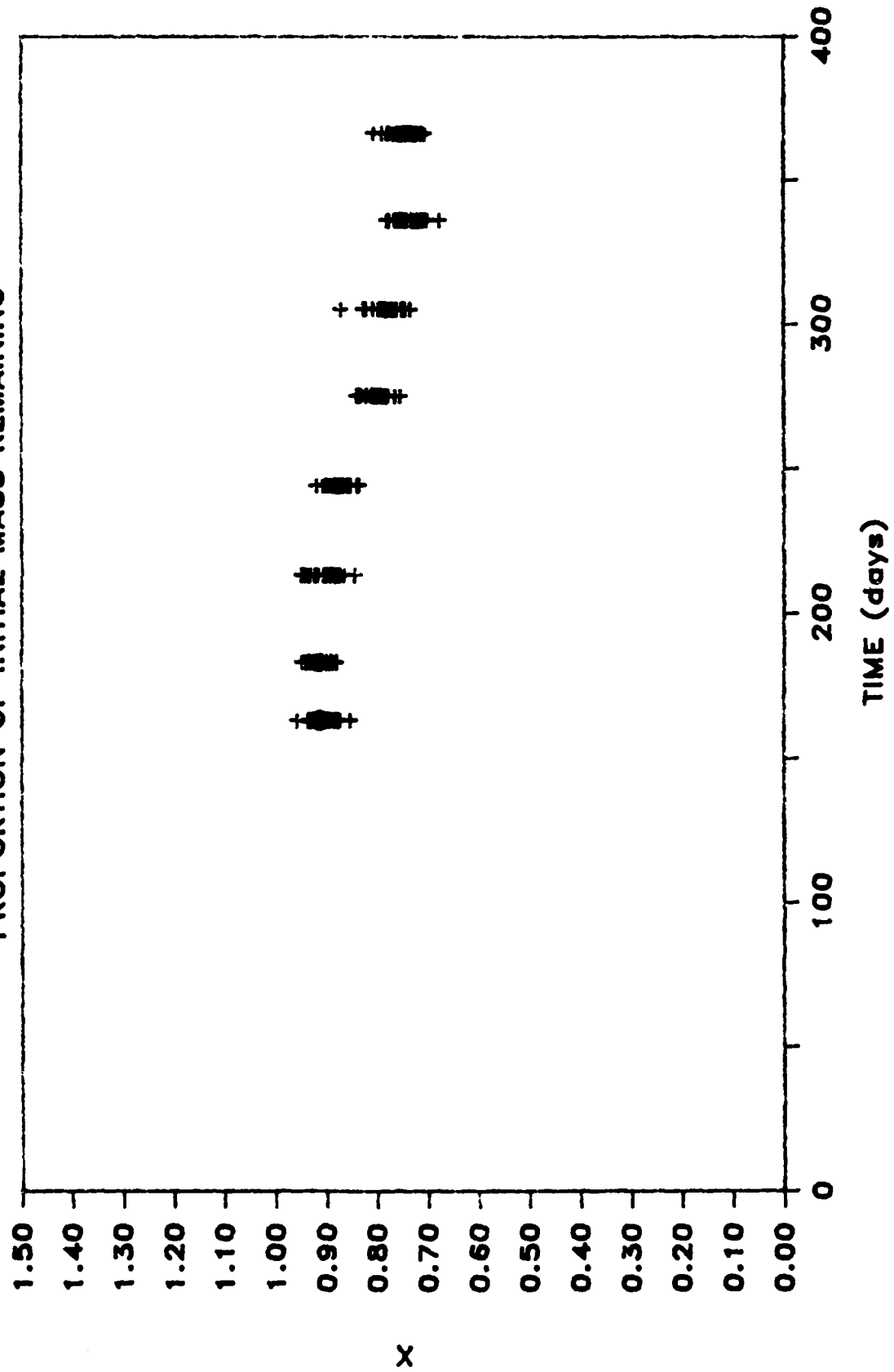
TOTAL MASS, INDIVIDUAL PINE FASCICLES



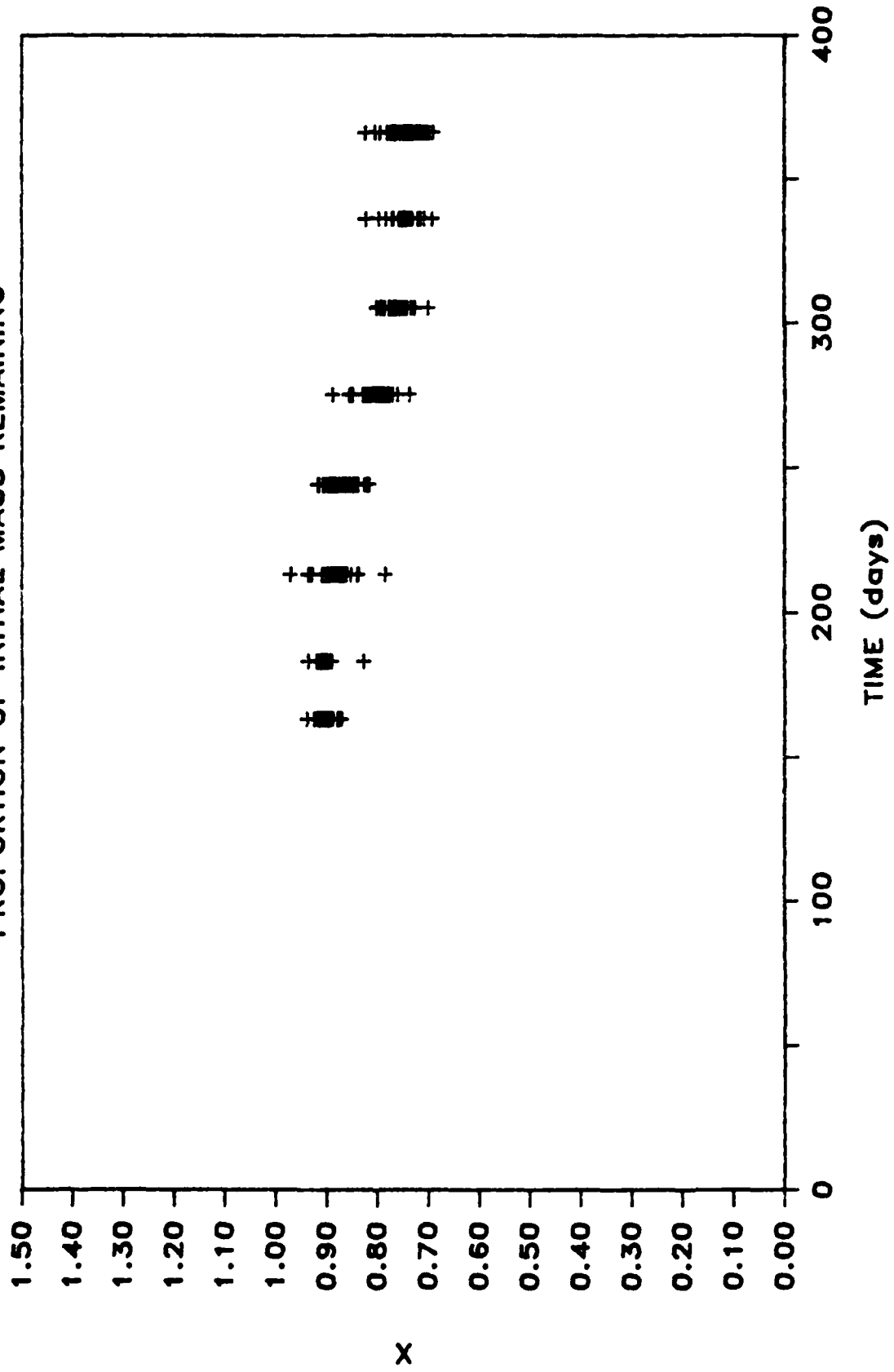
PINE FASCICLES, GROUND PLANTATION PROPORTION OF INITIAL MASS REMAINING



PINE FASCICLES, ANTENNA PLANTATION PROPORTION OF INITIAL MASS REMAINING

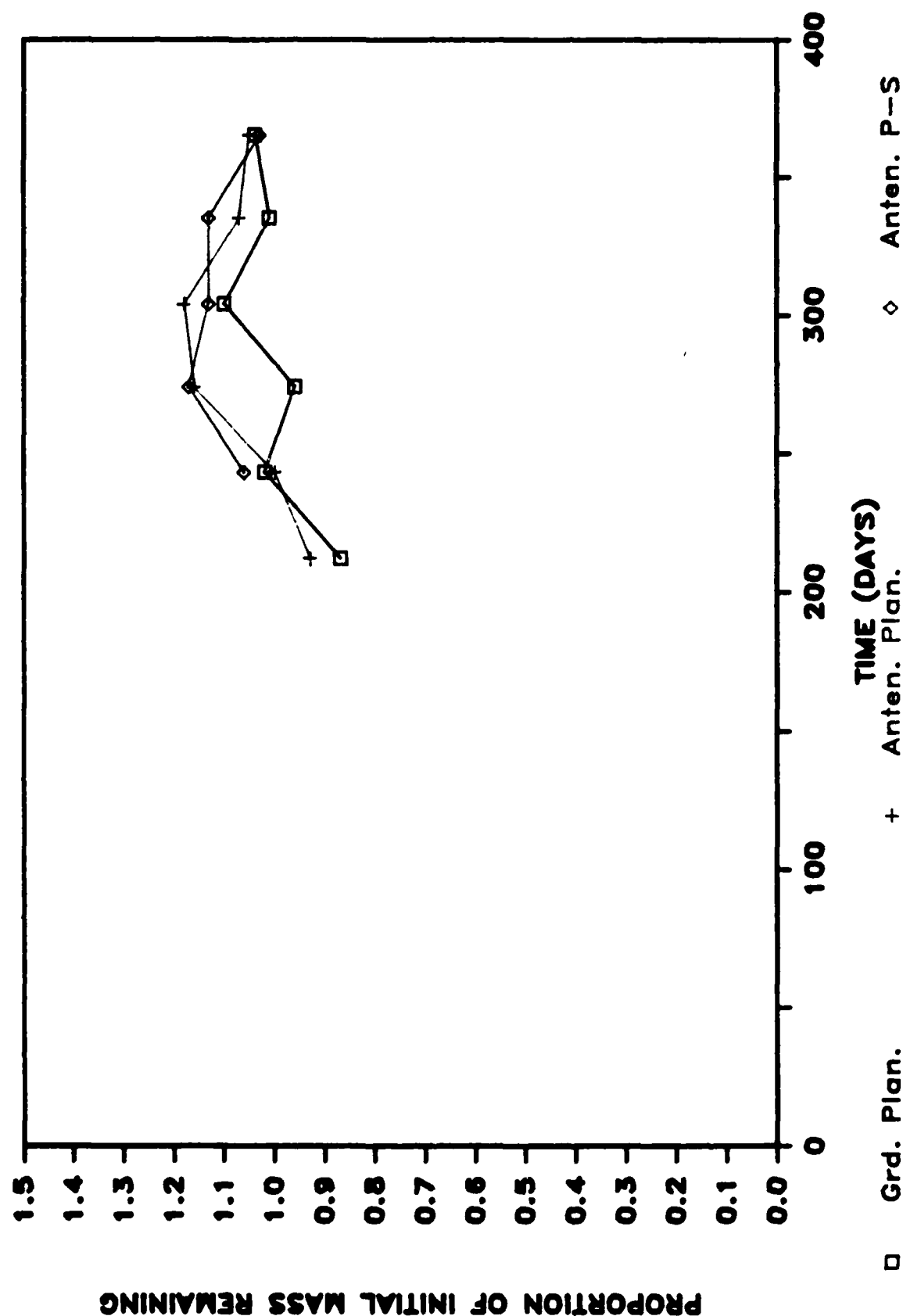


PINE FASCICLES, ANTENNA POLE-STAND PROPORTION OF INITIAL MASS REMAINING



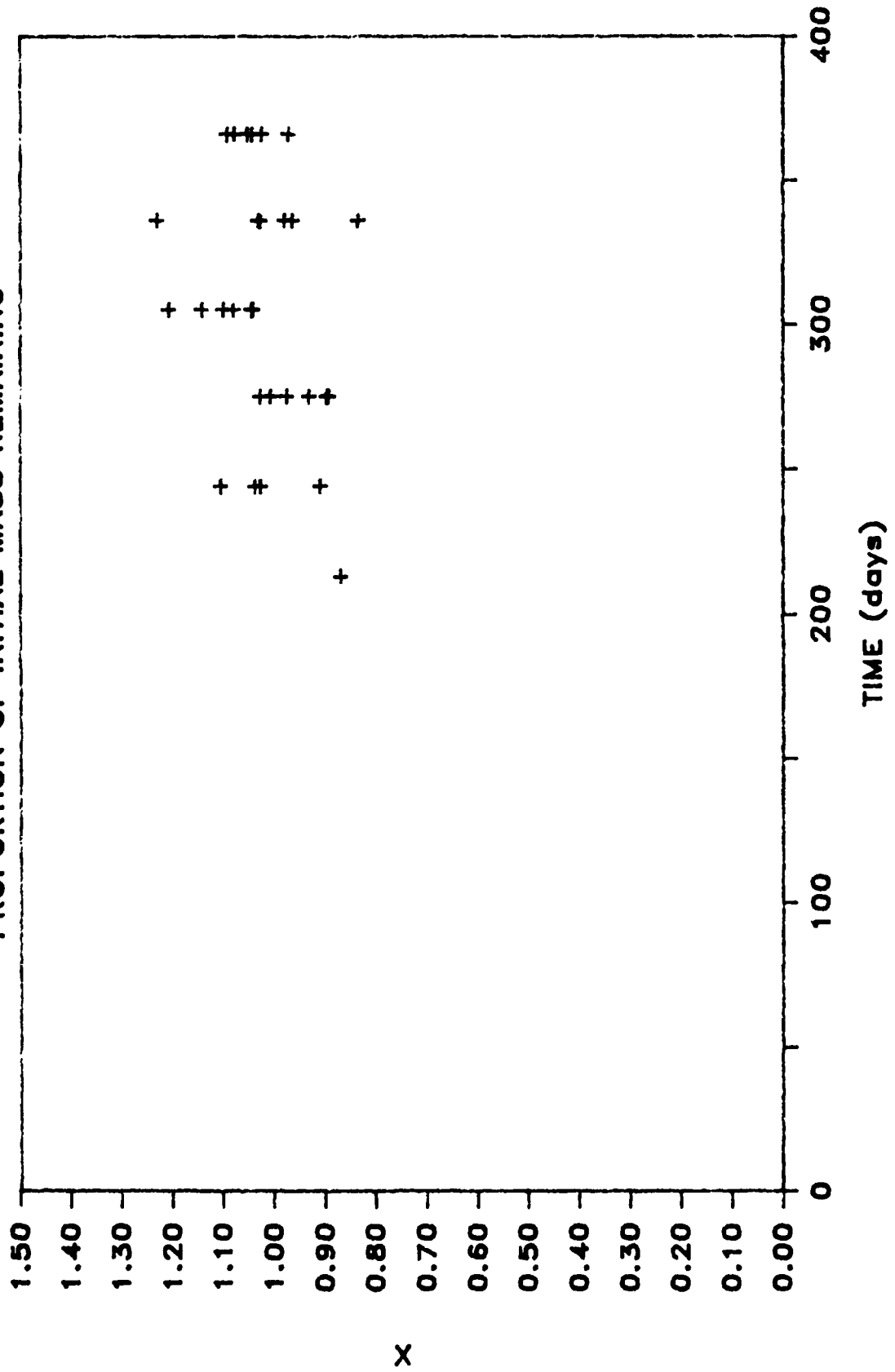
- Figure 9. Summary of nitrogen mass changes in pine litter at all three study locations between 1 December, 1983, and 1 December, 1984.
- Figure 10. Nitrogen mass changes in pine litter on the Ground site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 11. Nitrogen mass changes in pine litter on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 12. Nitrogen mass changes in pine litter in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.
- Figure 13. Summary of phosphorus mass changes in pine litter at all three study locations between 1 December, 1983, and 1 December, 1984.
- Figure 14. Phosphorus mass changes in pine litter on the Ground site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 15. Phosphorus mass changes in pine litter on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 16. Phosphorus mass changes in pine litter in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.
- Figure 17. Summary of potassium mass changes in pine litter at all three study locations between 1 December, 1983, and 1 December, 1984.
- Figure 18. Potassium mass changes in pine litter on the Ground site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 19. Potassium mass changes in pine litter on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 20. Potassium mass changes in pine litter in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.
- Figure 21. Summary of calcium mass changes in pine litter at all three study locations between 1 December, 1983, and 1 December, 1984.
- Figure 22. Calcium mass changes in pine litter on the Ground site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 23. Calcium mass changes in pine litter on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 24. Calcium mass changes in pine litter in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.
- Figure 25. Summary of magnesium mass changes in pine litter at all three study locations between 1 December, 1983, and 1 December, 1984.
- Figure 26. Magnesium mass changes in pine litter on the Ground site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 27. Magnesium mass changes in pine litter on the Antenna site plantation between 1 December, 1983, and 1 December, 1984.
- Figure 28. Magnesium mass changes in pine litter in the Antenna site pole-stand between 1 December, 1983, and 1 December, 1984.

N IN BULK LITTER ENVELOPES



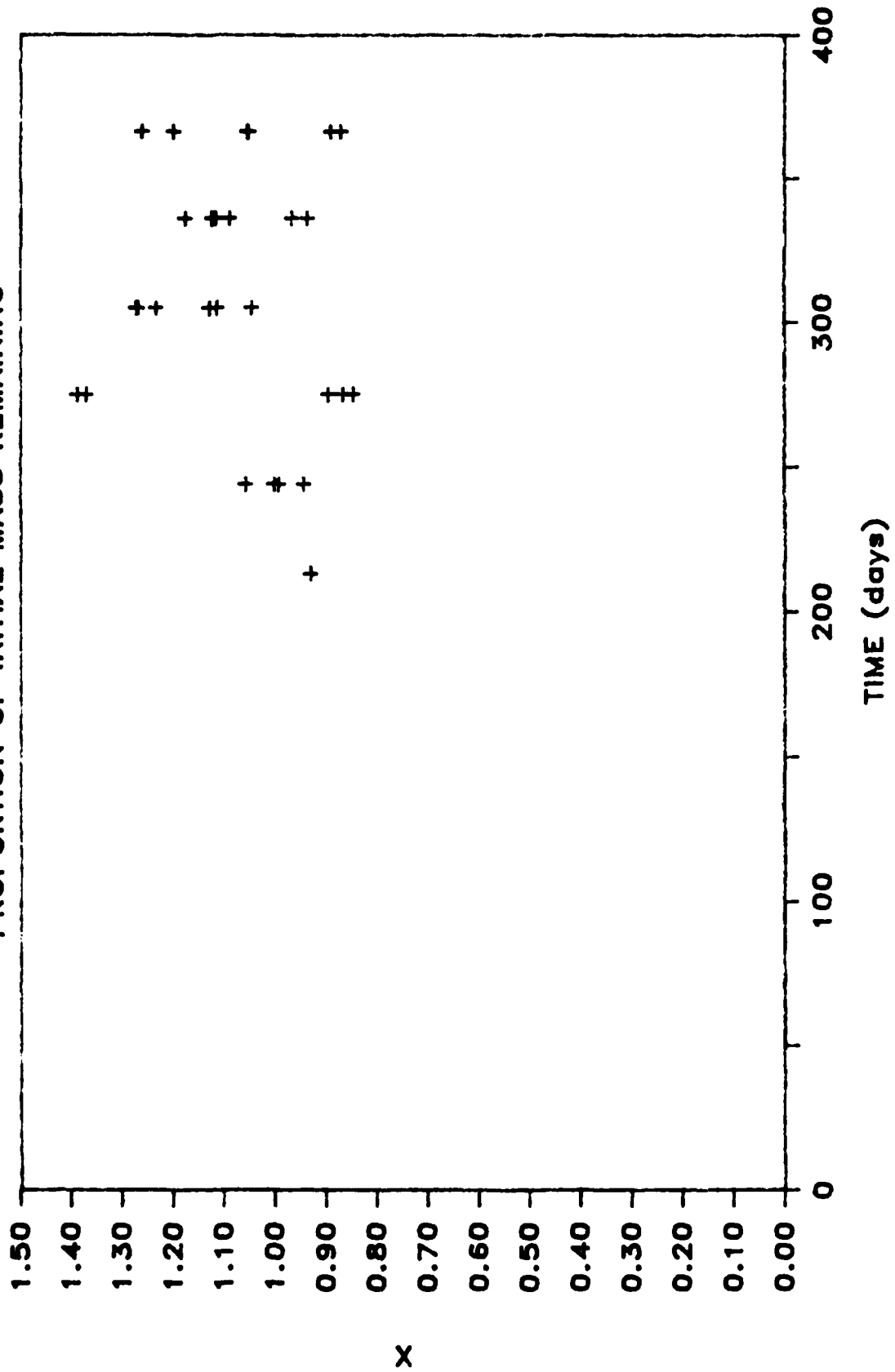
N IN PINE LITTER, GROUND PLANTATION

PROPORTION OF INITIAL MASS REMAINING



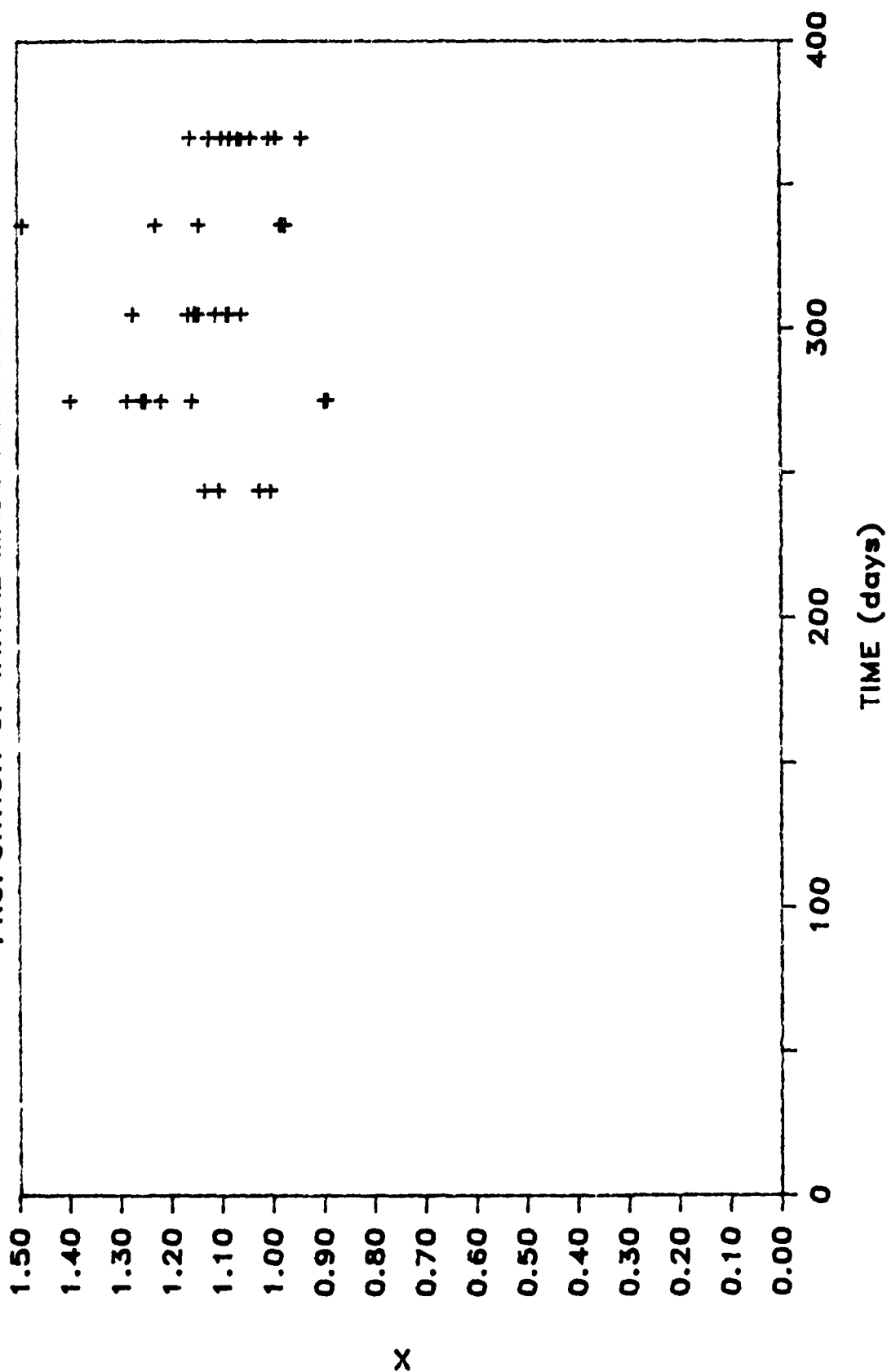
N IN PINE LITTER, ANTENNA PLANTATION

PROPORTION OF INITIAL MASS REMAINING



N IN PINE LITTER, ANTENNA POLE--STAND

PROPORTION OF INITIAL MASS REMAINING



P IN BULK PINE LITTER ENVELOPES

28

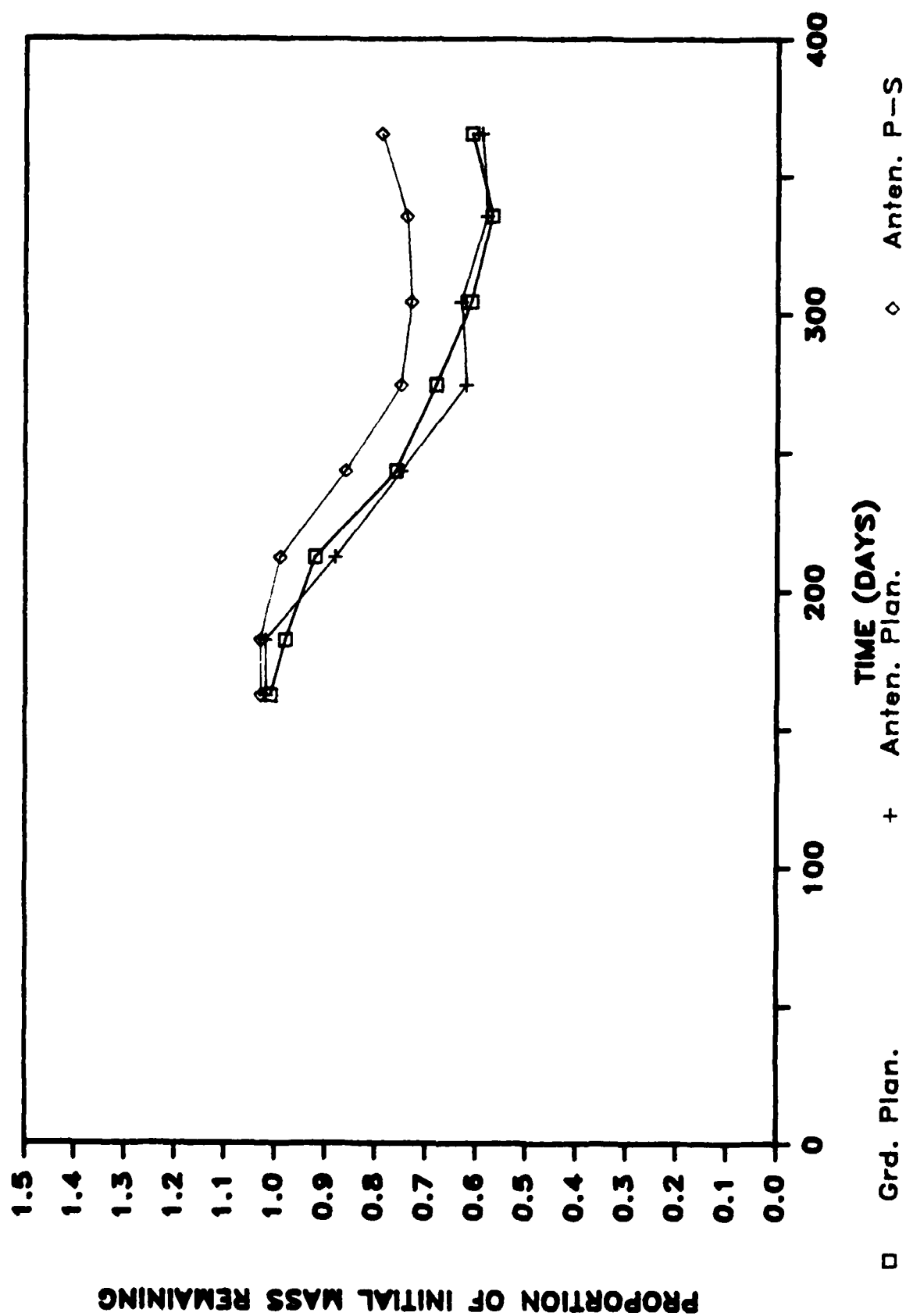
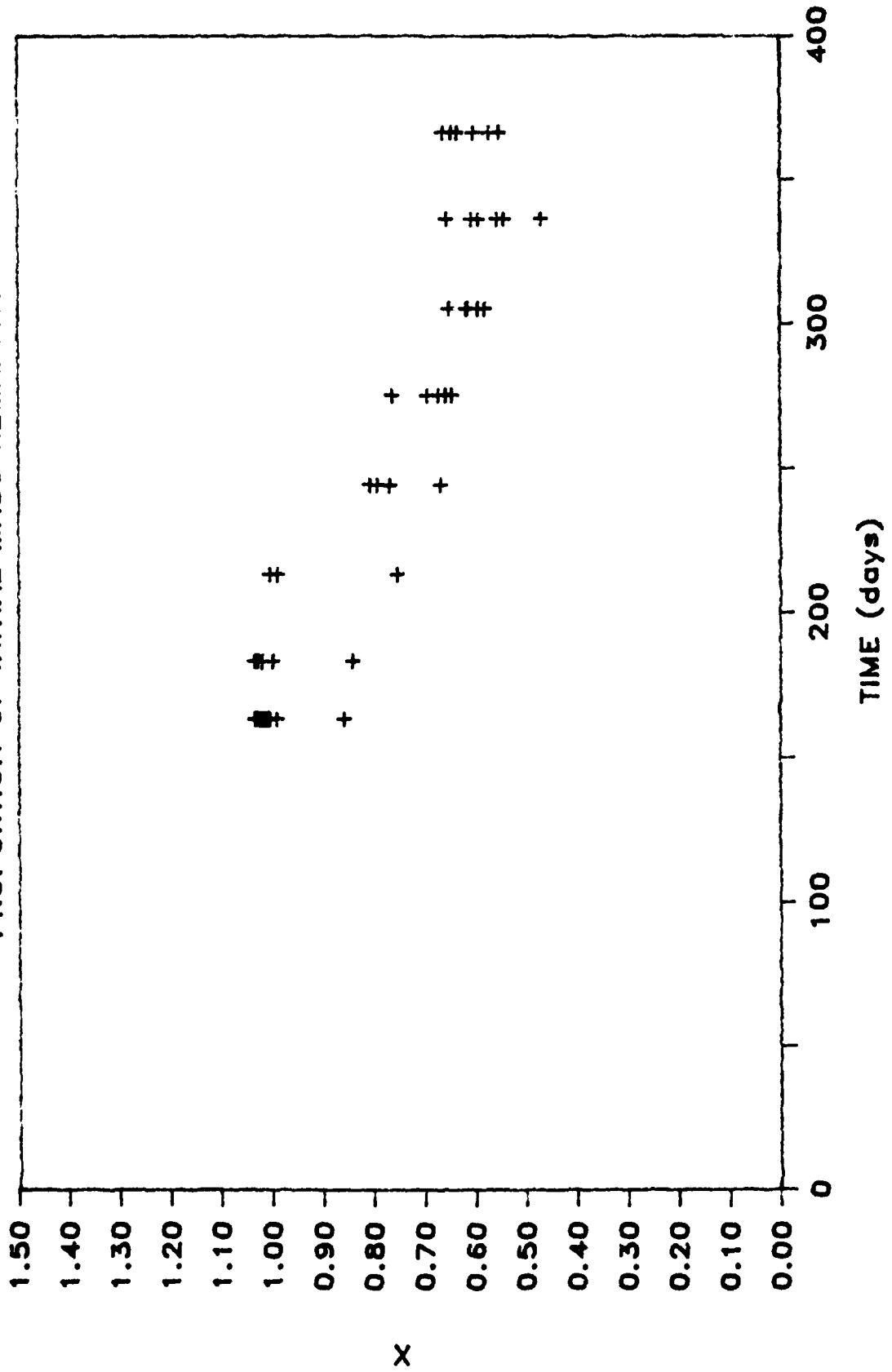


Figure 1 displays a sequence of 15 panels illustrating the evolution of a 2D spatial pattern. The pattern starts as a noisy, irregular distribution of black dots on a white background and gradually evolves through various stages of self-organization, eventually forming a highly regular, hexagonal lattice structure. The panels are labeled with numbers 1 through 15 on the right side.

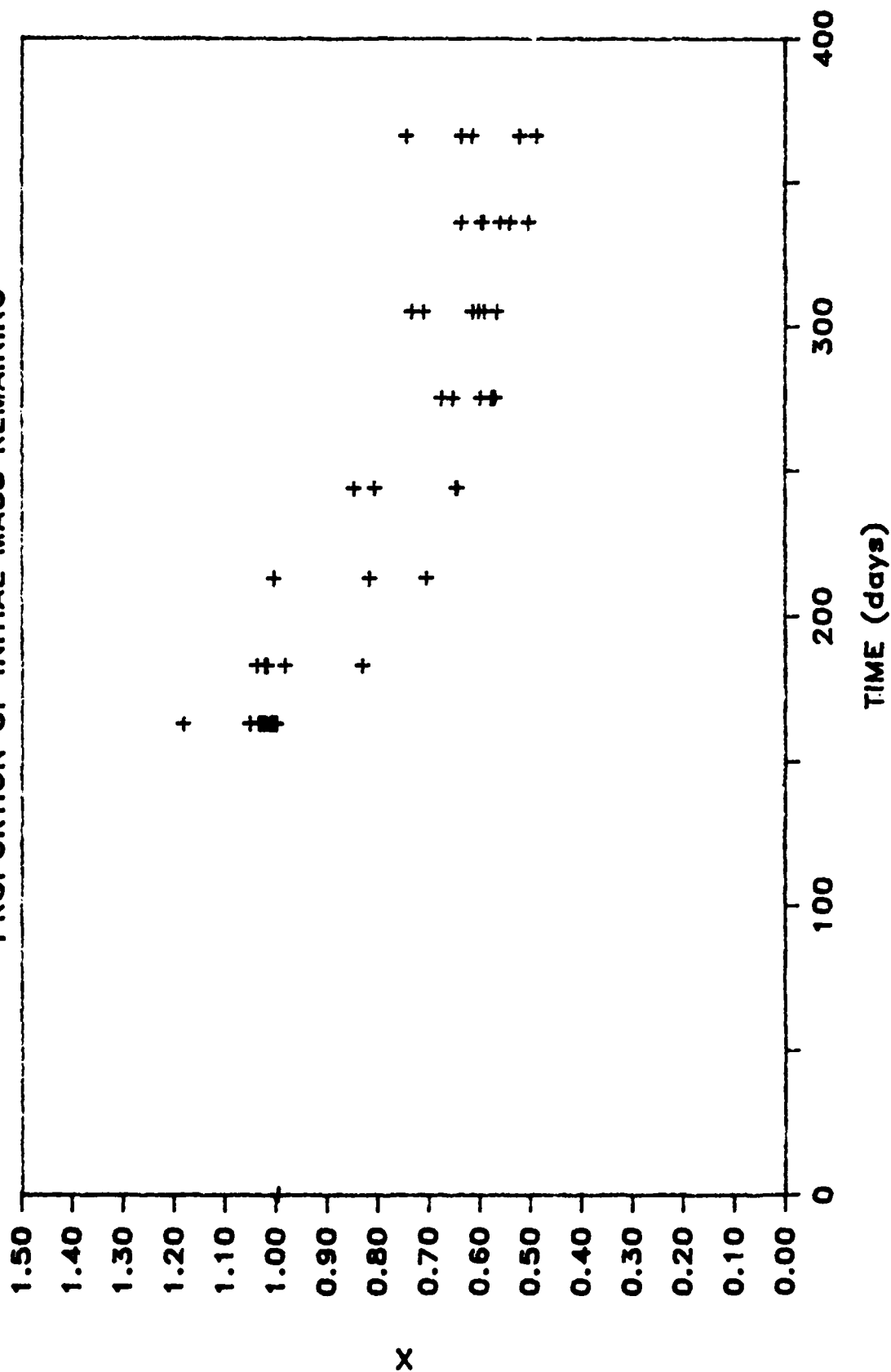
P IN PINE LITTER, GROUND PLANTATION

PROPORTION OF INITIAL MASS REMAINING

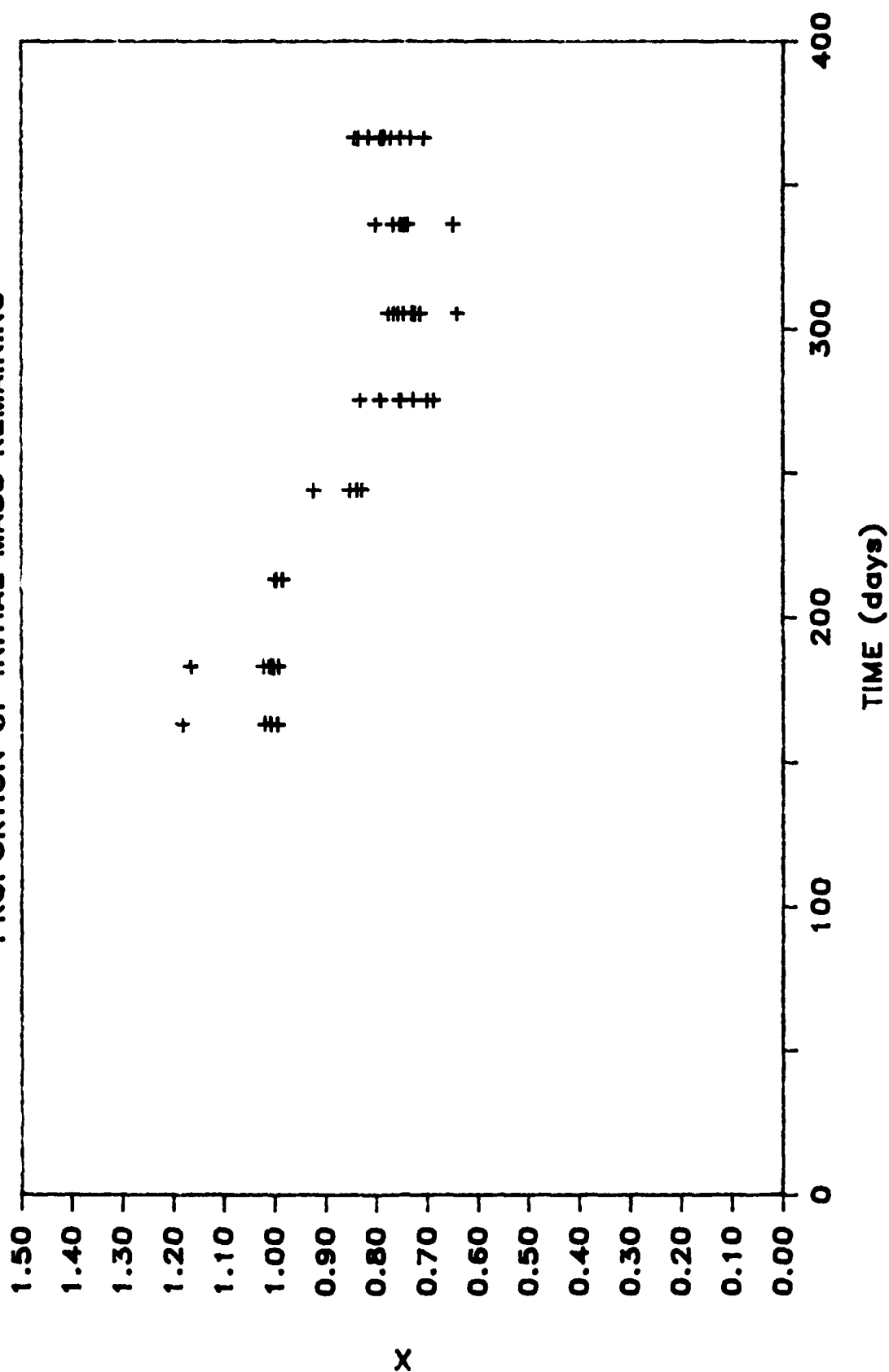


P IN PINE LITTER, ANTENNA PLANTATION

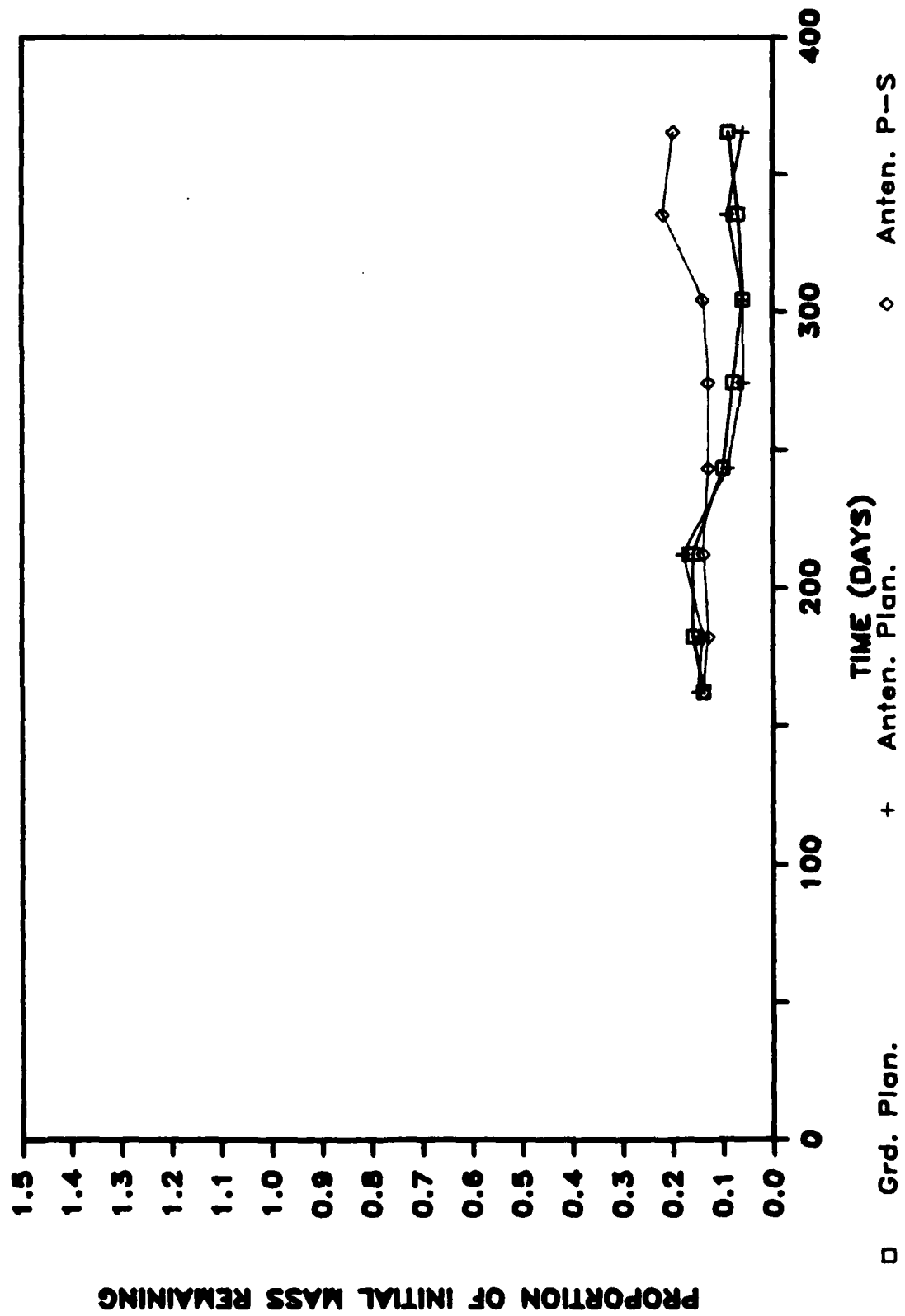
PROPORTION OF INITIAL MASS REMAINING



P IN PINE LITTER, ANTENNA POLE-STAND PROPORTION OF INITIAL MASS REMAINING

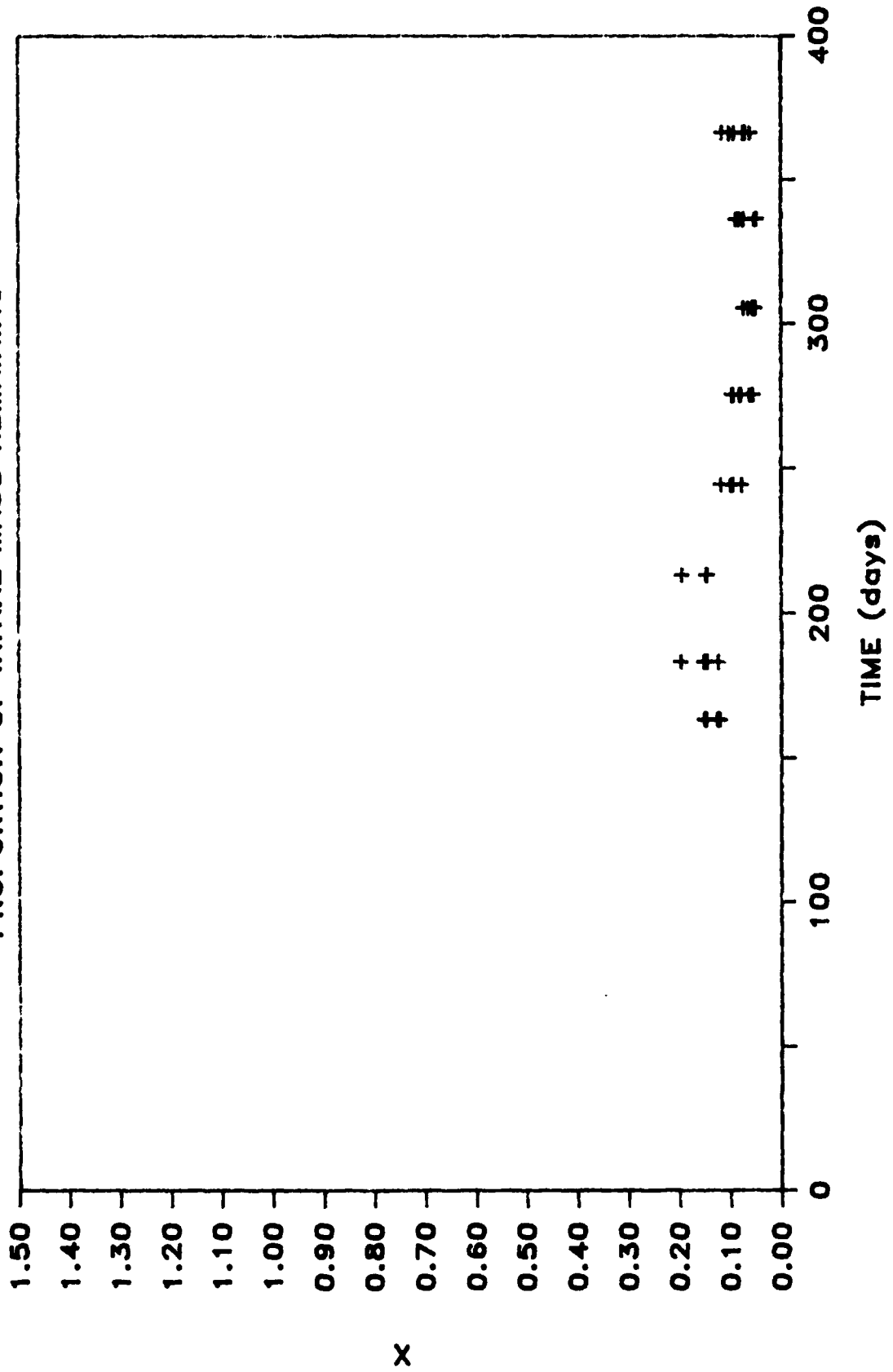


K IN BULK PINE LITTER ENVELOPES



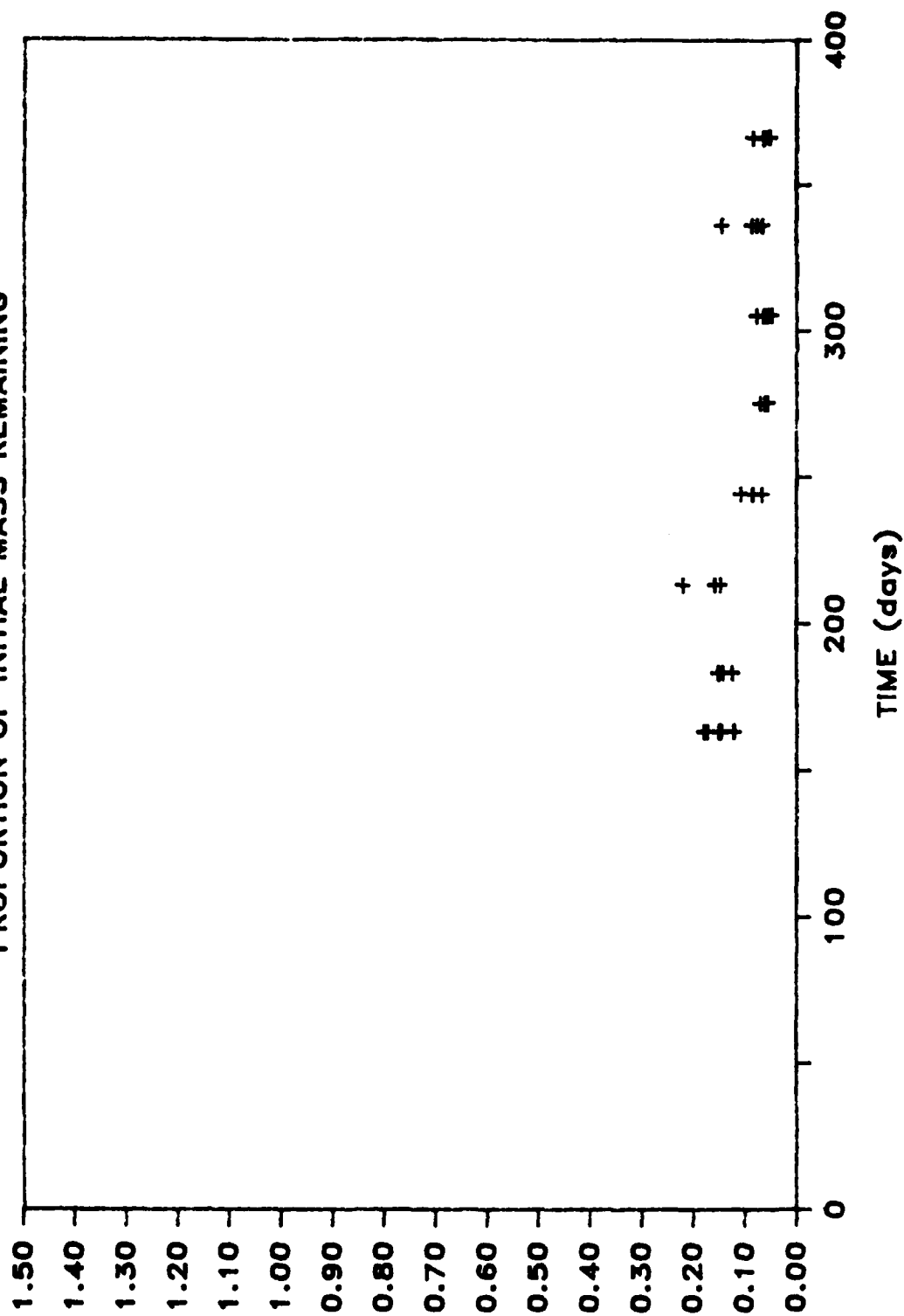
K IN PINE LITTER, GROUND PLANTATION

PROPORTION OF INITIAL MASS REMAINING



K IN PINE LITTER, ANTENNA PLANTATION

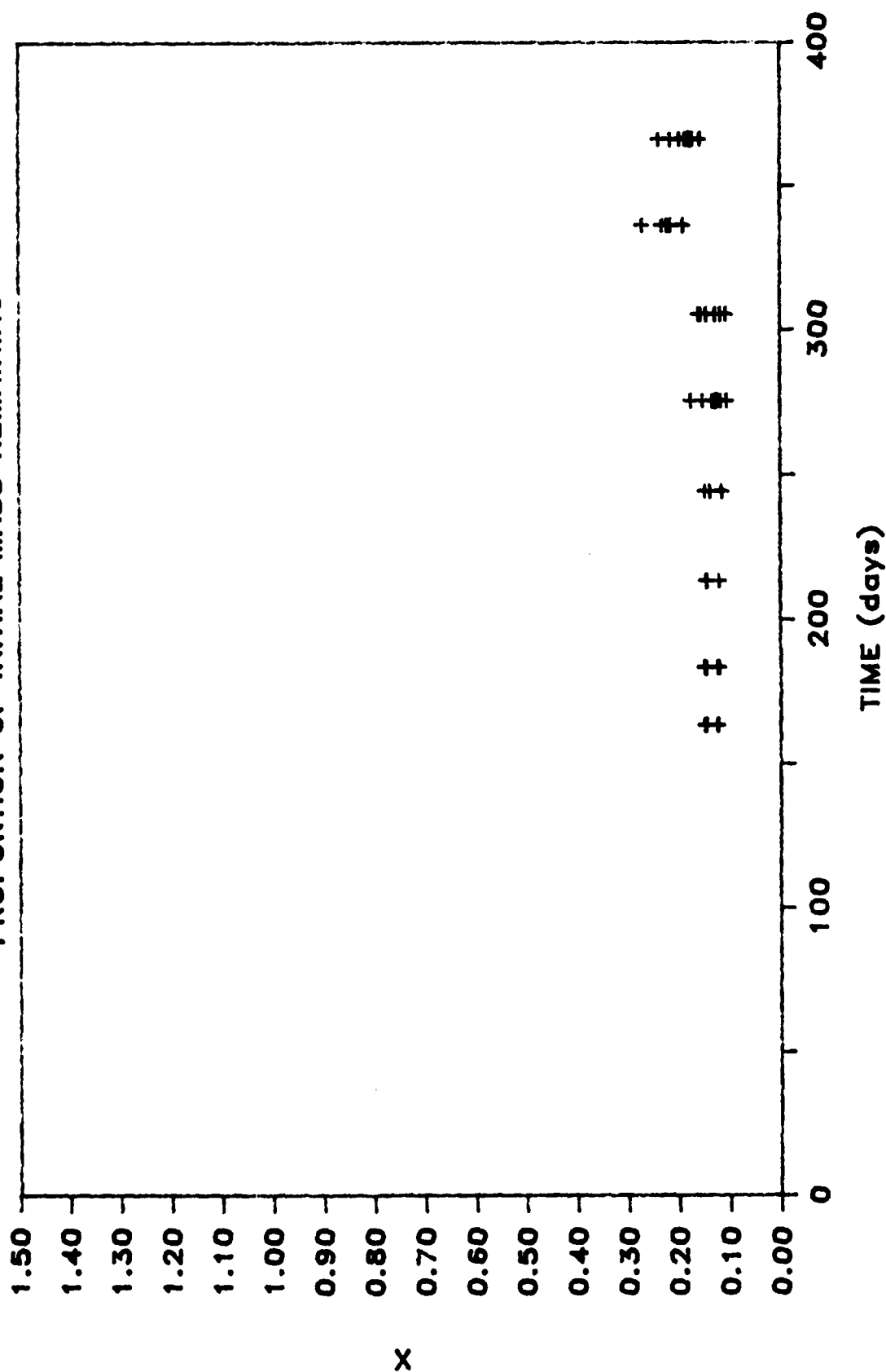
PROPORTION OF INITIAL MASS REMAINING



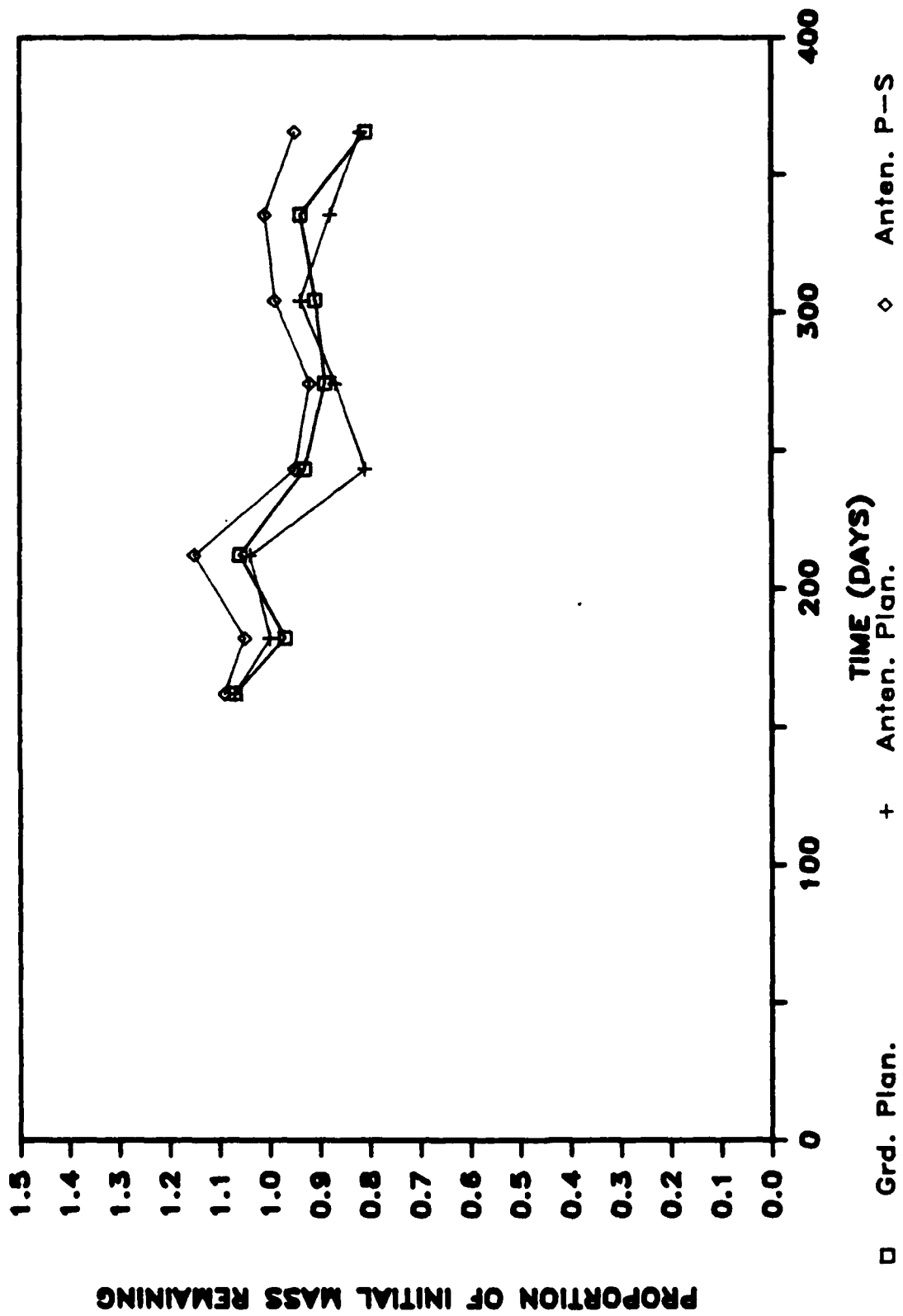
x

K IN PINE LITTER, ANTENNA POLE-STAND

PROPORTION OF INITIAL MASS REMAINING

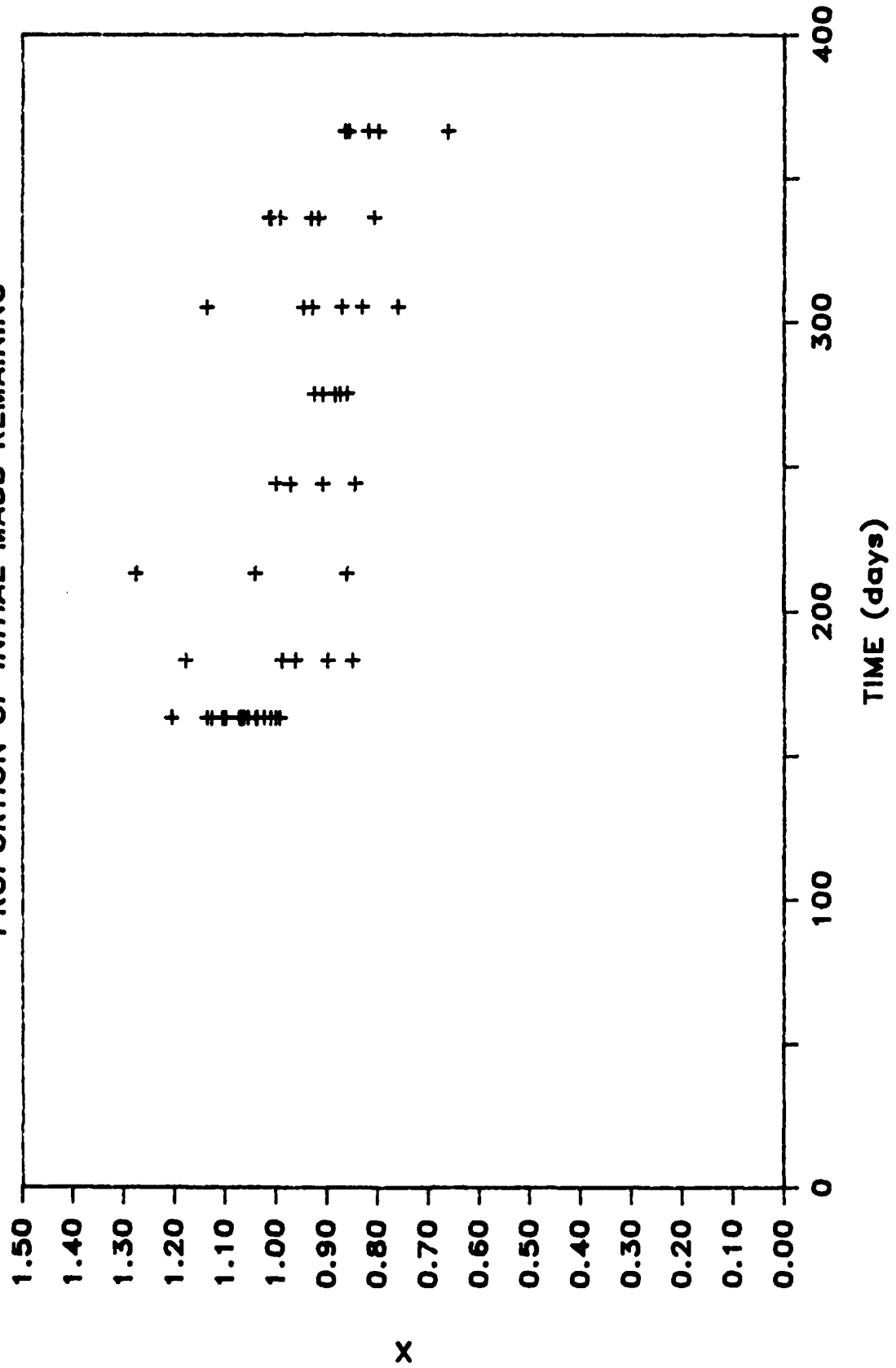


Ca IN BULK PINE LITTER ENVELOPES



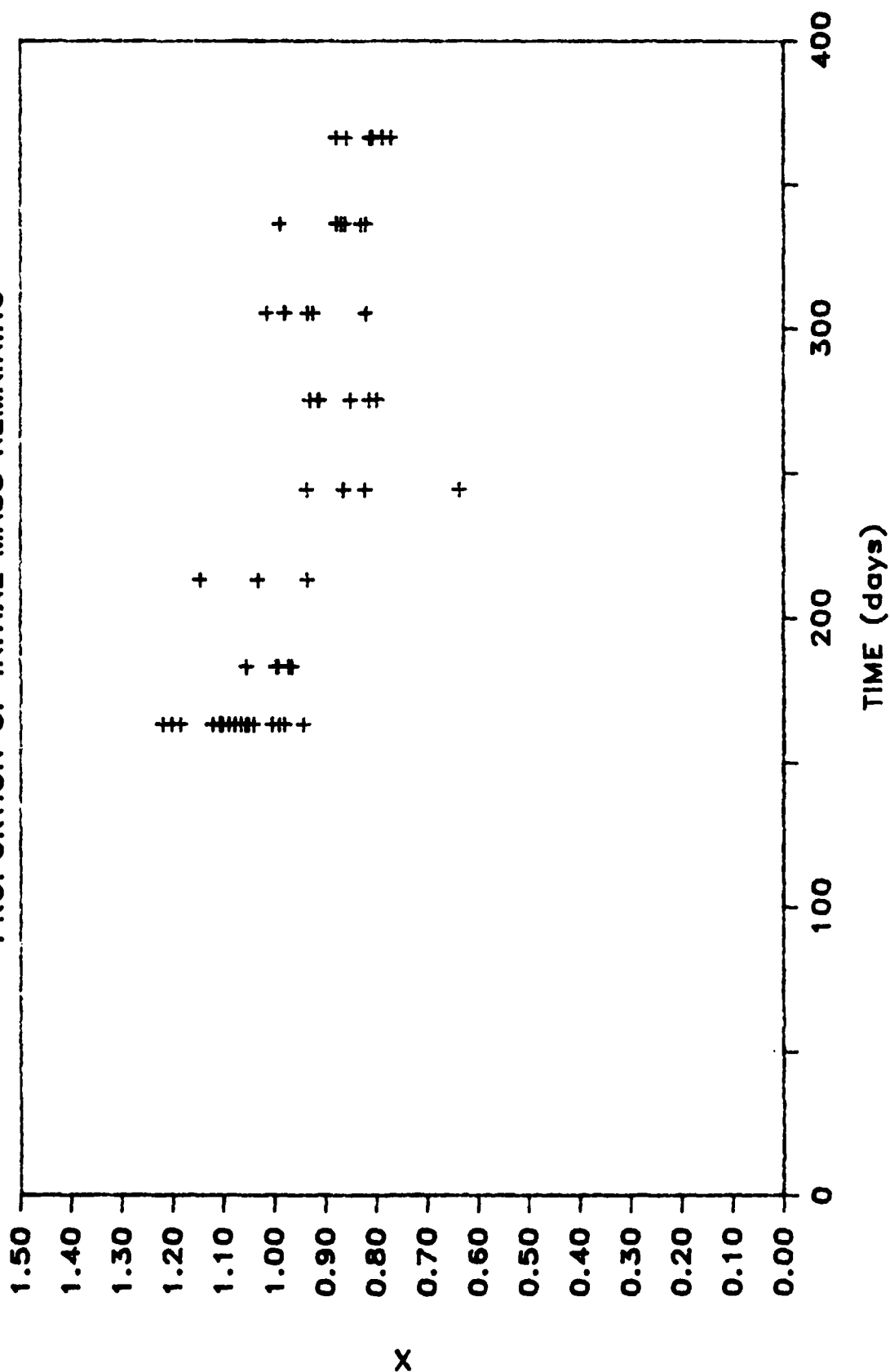
Ca IN PINE LITTER, GROUND PLANTATION

PROPORTION OF INITIAL MASS REMAINING



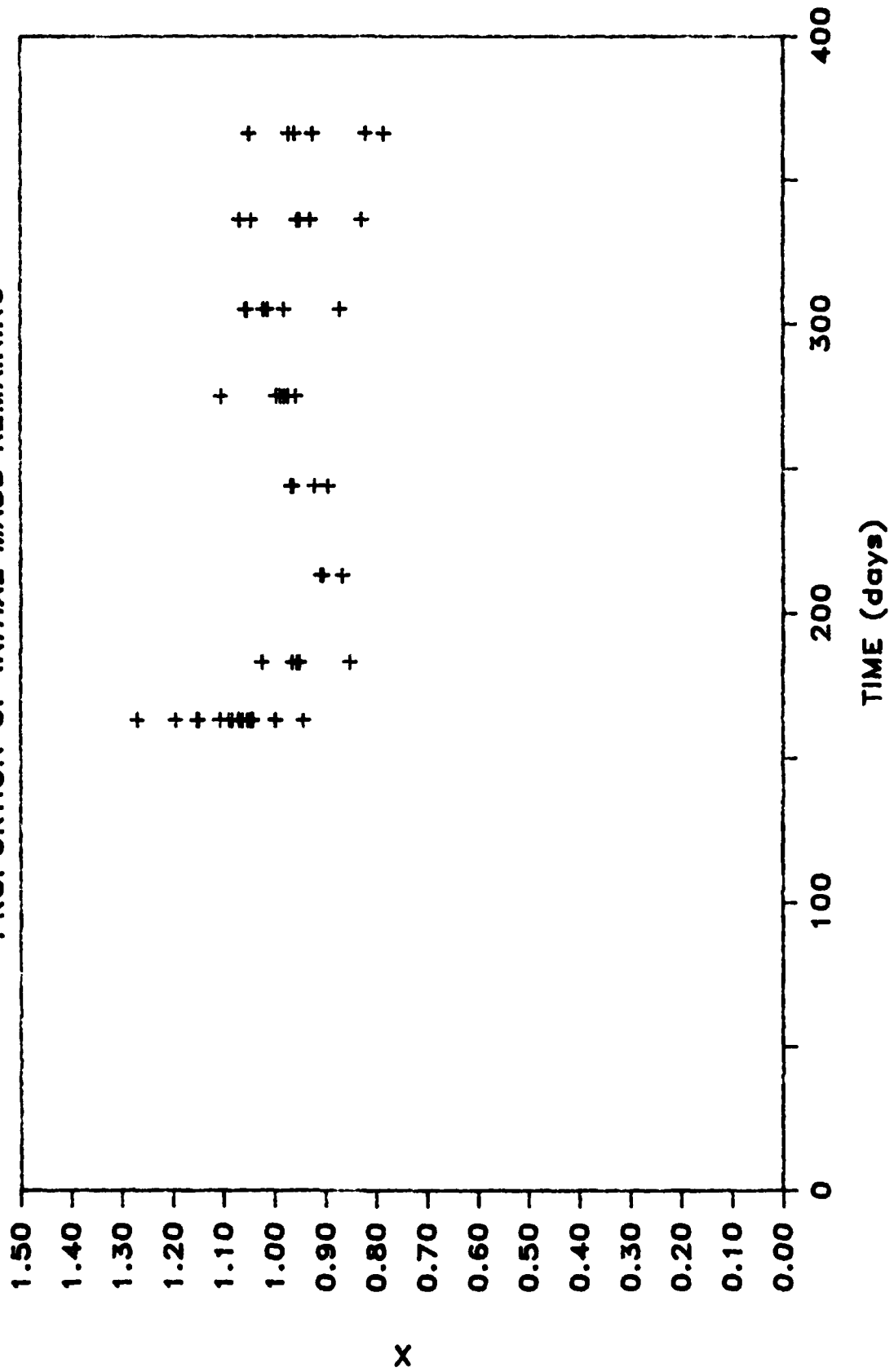
Ca IN PINE LITTER, ANTENNA PLANTATION

PROPORTION OF INITIAL MASS REMAINING

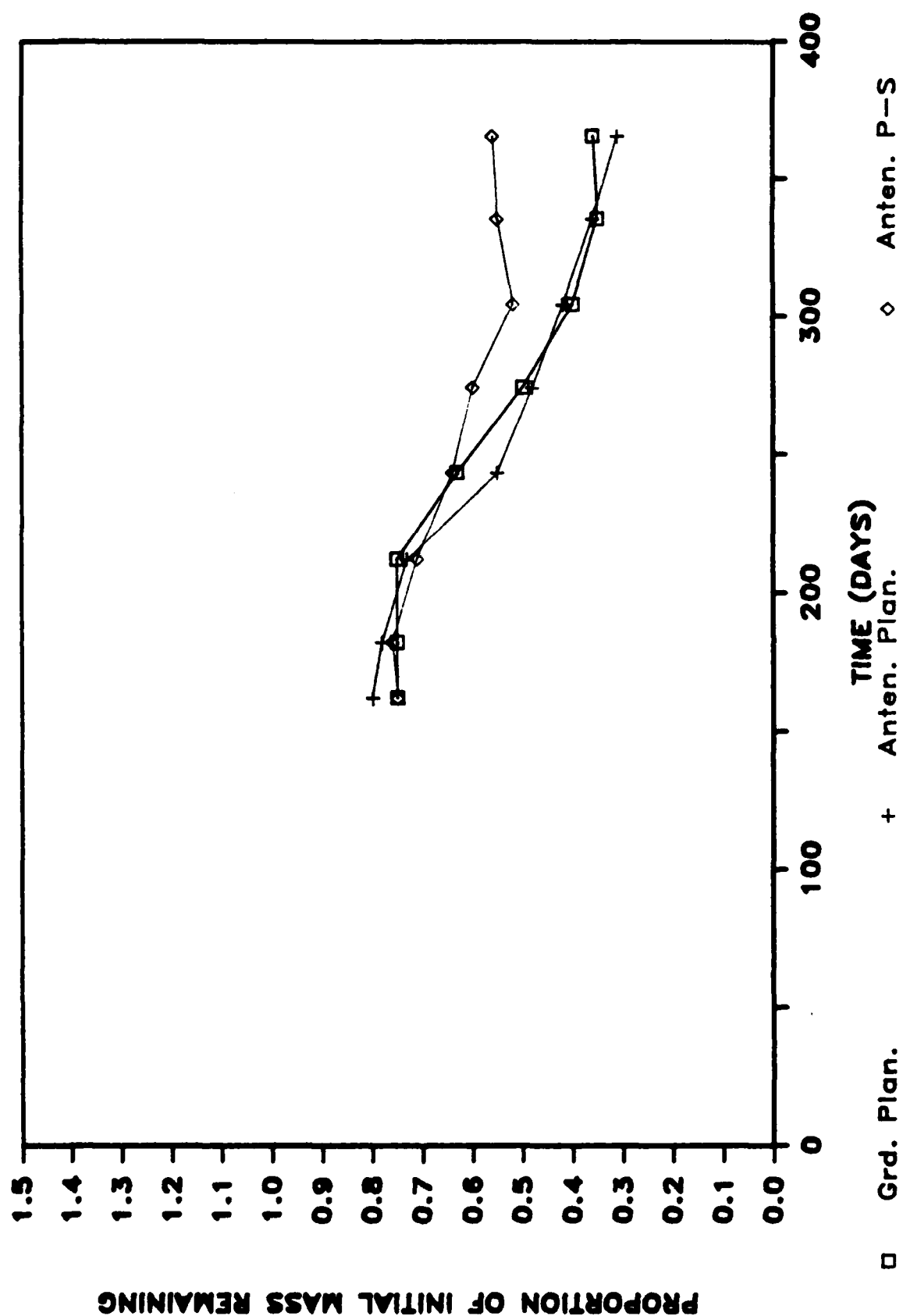


Ca IN PINE LITTER, ANTENNA POLE-STAND

PROPORTION OF INITIAL MASS REMAINING

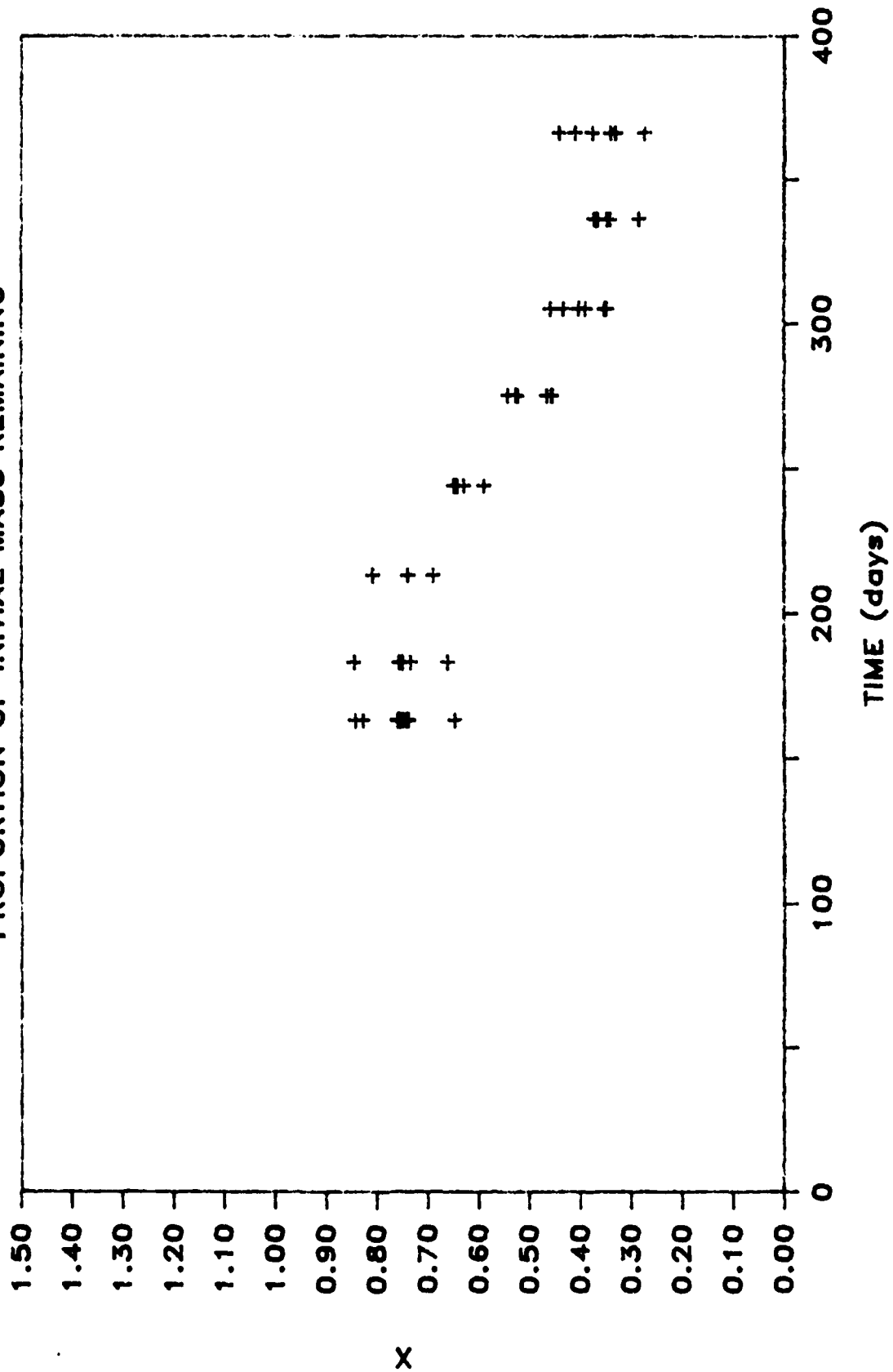


Mg IN BULK PINE LITTER ENVELOPES



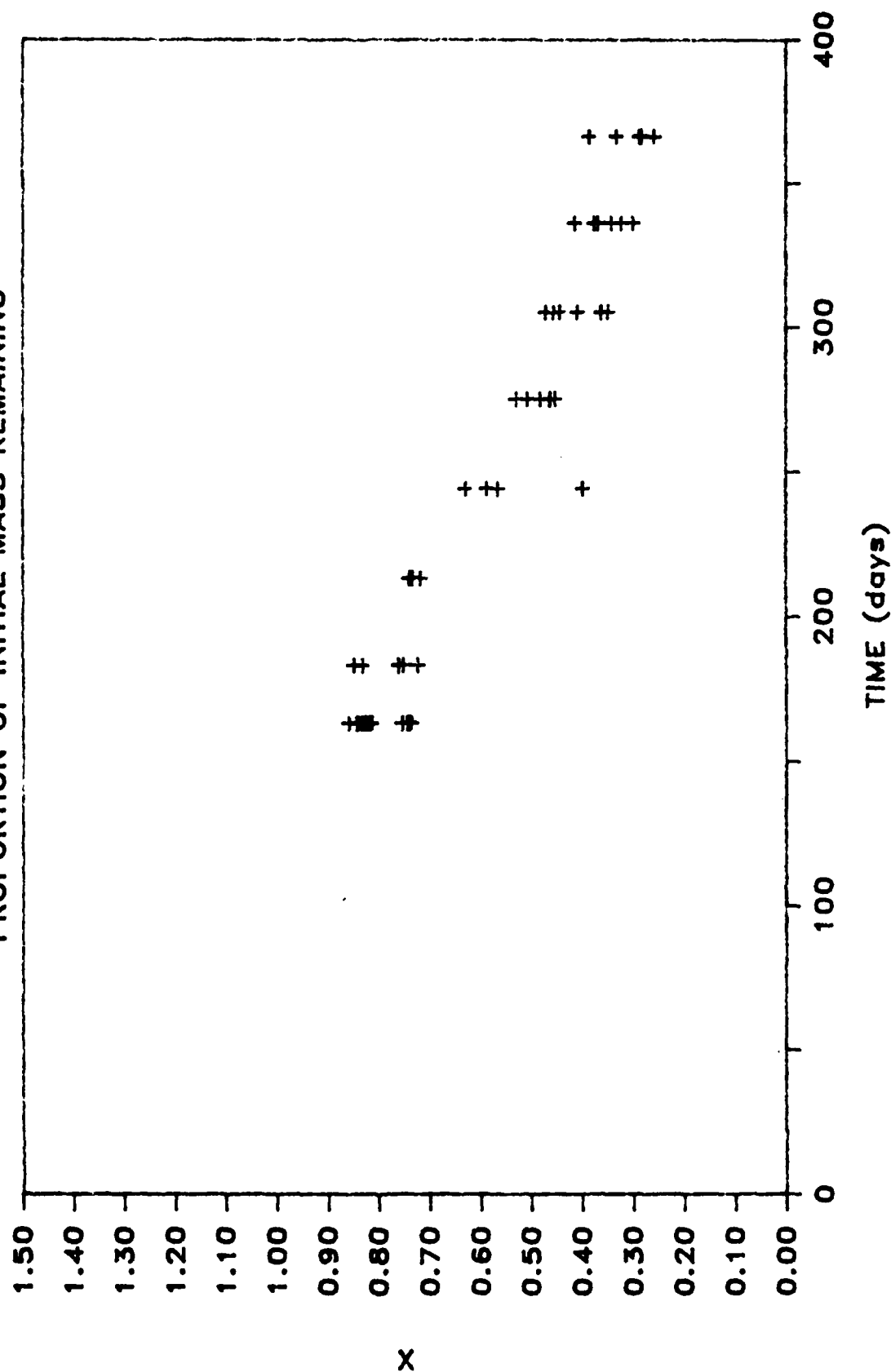
Mg IN PINE LITTER, GROUND PLANTATION

PROPORTION OF INITIAL MASS REMAINING



Mg IN PINE LITTER, ANTENNA PLANTATION

PROPORTION OF INITIAL MASS REMAINING



Mg IN PINE LITTER, ANTENNA POLE-STAND PROPORTION OF INITIAL MASS REMAINING

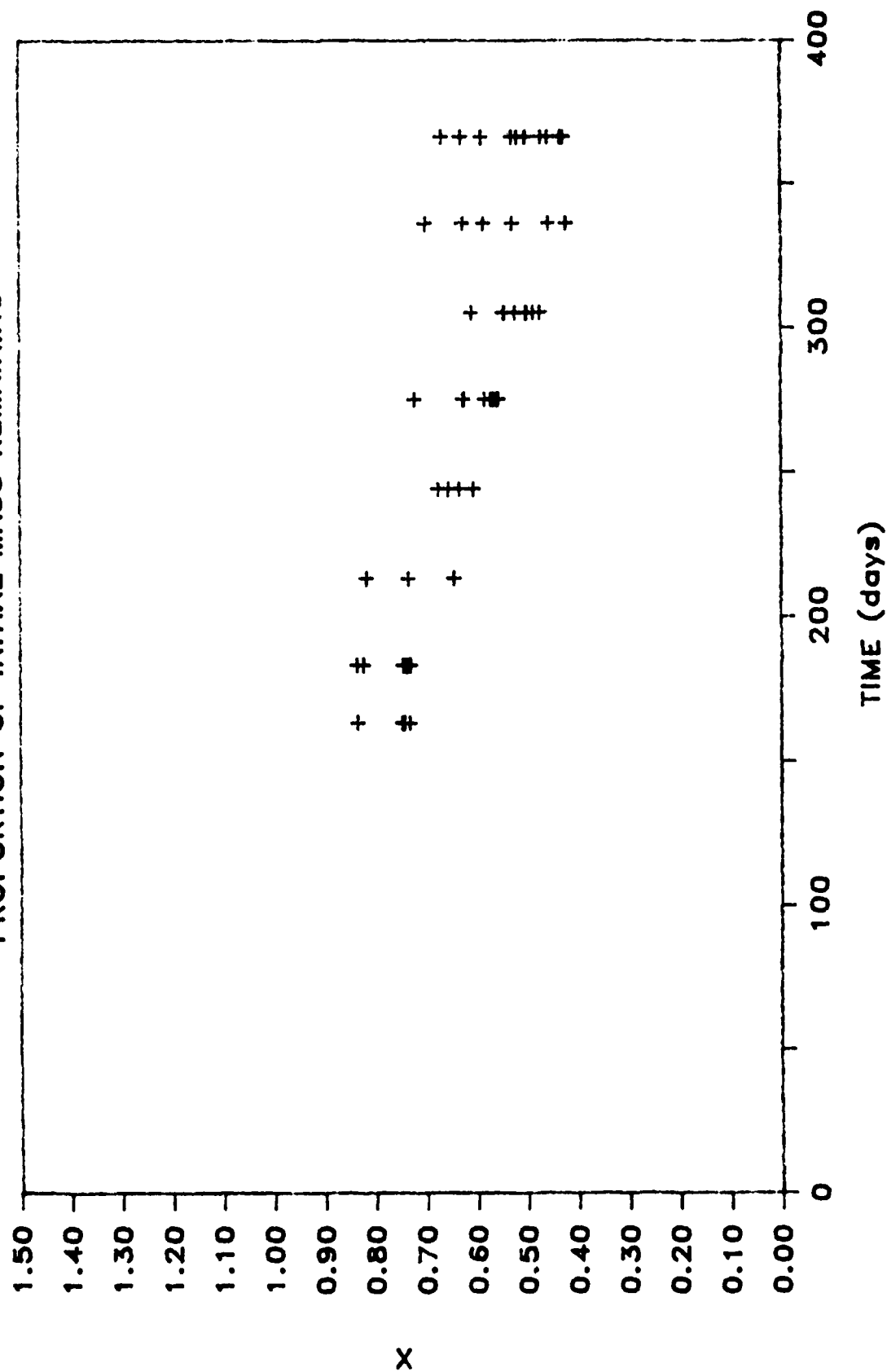


Table 8. Characteristics of single exponential models^a tested for fit with first year overall mass loss from fresh-fallen pine foliar litter at the overhead and ground sites.

	Fascicles ^b			Bulk Samples ^c		
	Antenna Site		Ground Site	Antenna Site		Ground Site
	Pole-stand	Plantation	Plantation	Pole-stand	Plantation	Plantation
$X = e^{-kt} ; k_i = 7.50 \times 10^{-4}$						
$k (x 10^{-4})$	7.53	7.06	7.05	7.42	7.01	7.05
$SD_k (x 10^{-5})^d$	1.0	1.2	1.1	1.7	2.1	2.4
df ^e	262	280	275	53	53	53
CI _k upper (x 10 ⁻⁴) ^f	7.73	7.30	7.27	7.76	7.43	7.54
CI _k lower (x 10 ⁻⁴)	7.33	6.82	6.83	7.08	6.59	6.58
$X = e^{-k(t-l)} ; k_i = 7.5 \times 10^{-4} , l_i = 100$						
$k (x 10^{-3})$	1.10	1.13	1.09	1.07	1.05	1.09
$SD_k (x 10^{-5})$	3.4	2.8	3.7	4.4	4.9	5.6
l	88	95	88	87	85	91
SD_l	5.5935	3.7271	5.2119	7.6570	7.7056	8.1426
df	261	279	274	52	52	52
CI _k upper (x 10 ⁻³)	1.17	1.19	1.16	1.16	1.15	1.20
CI _k lower (x 10 ⁻³)	1.03	1.07	1.02	0.98	0.95	0.98
CI _l upper	99	102	98	102	100	107
CI _l lower	77	88	78	72	70	75
$X = e^{-k(t-89)} ; k_i = 7.50 \times 10^{-4} , l_i = 100$						
$k (x 10^{-3})$	1.11	1.10	1.10	1.09	1.07	1.08
$SD_k (x 10^{-5})$	1.3	1.3	1.6	1.7	2.2	2.5
df	262	280	275	53	53	53
CI _k upper (x 10 ⁻³)	1.14	1.13	1.13	1.12	1.11	1.13
CI _k lower (x 10 ⁻³)	1.08	1.07	1.07	1.06	1.03	1.03

a/ Models were derived using BMDP4R, for derivative-free nonlinear regression. Mass loss is expressed as the proportion (X) of initial mass remaining at time of sampling (t); k is the decomposition rate constant (k_i is the estimate of k used to initiate the iterative process); l is the lag period in days. Samples were retrieved monthly from mid-May through early December.

b/ Fascicle samples consist of bagged (9 in x 5 in, 3 mm mesh, nylon envelopes), individually identified, perfectly-formed fascicles.

c/ Bulk samples consist of 10 g of bagged (12 in x 8 in, 3 mm mesh, nylon envelopes) pine fascicles.

d/ asymptotic standard deviation

e/ degrees of freedom

f/ upper and lower bounds of confidence interval ($\alpha = 0.05$)

Figures 29 through 34.

Figure 29. Forms of single exponential model best fitting overall mass loss by bulk pine samples at the three study locations without a lag period.

Figure 30. Forms of single exponential model best fitting overall mass loss by individual pine fascicles at the three study locations without a lag period.

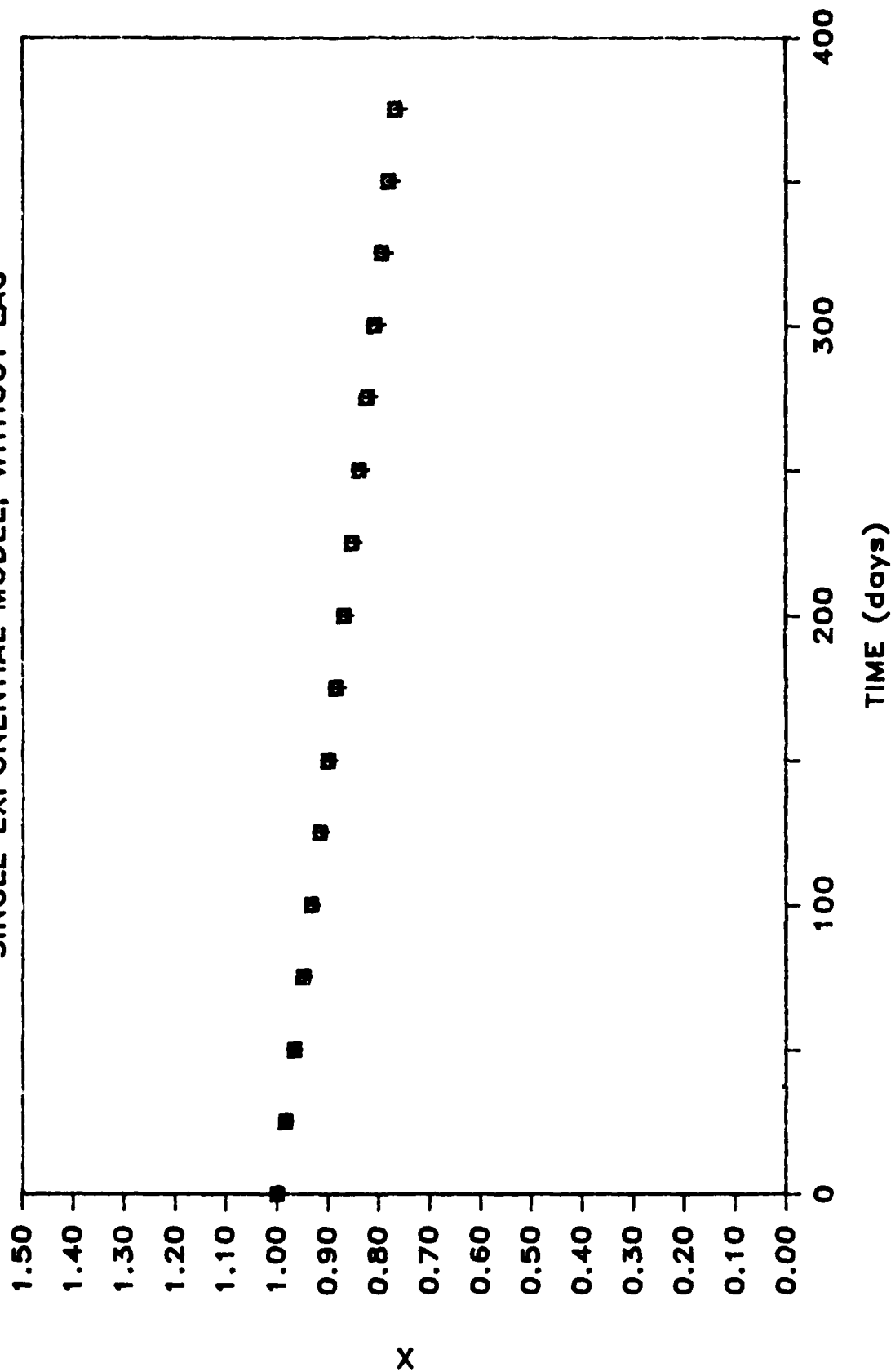
Figure 31. Forms of single exponential model best fitting overall mass loss by bulk pine samples at the three study locations with independently determined lag periods.

Figure 32. Forms of single exponential model best fitting overall mass loss by individual pine fascicles at the three study locations with independently determined lag periods.

Figure 33. Forms of single exponential model best fitting overall mass loss by bulk pine samples at the three study locations with lag period of 89 days.

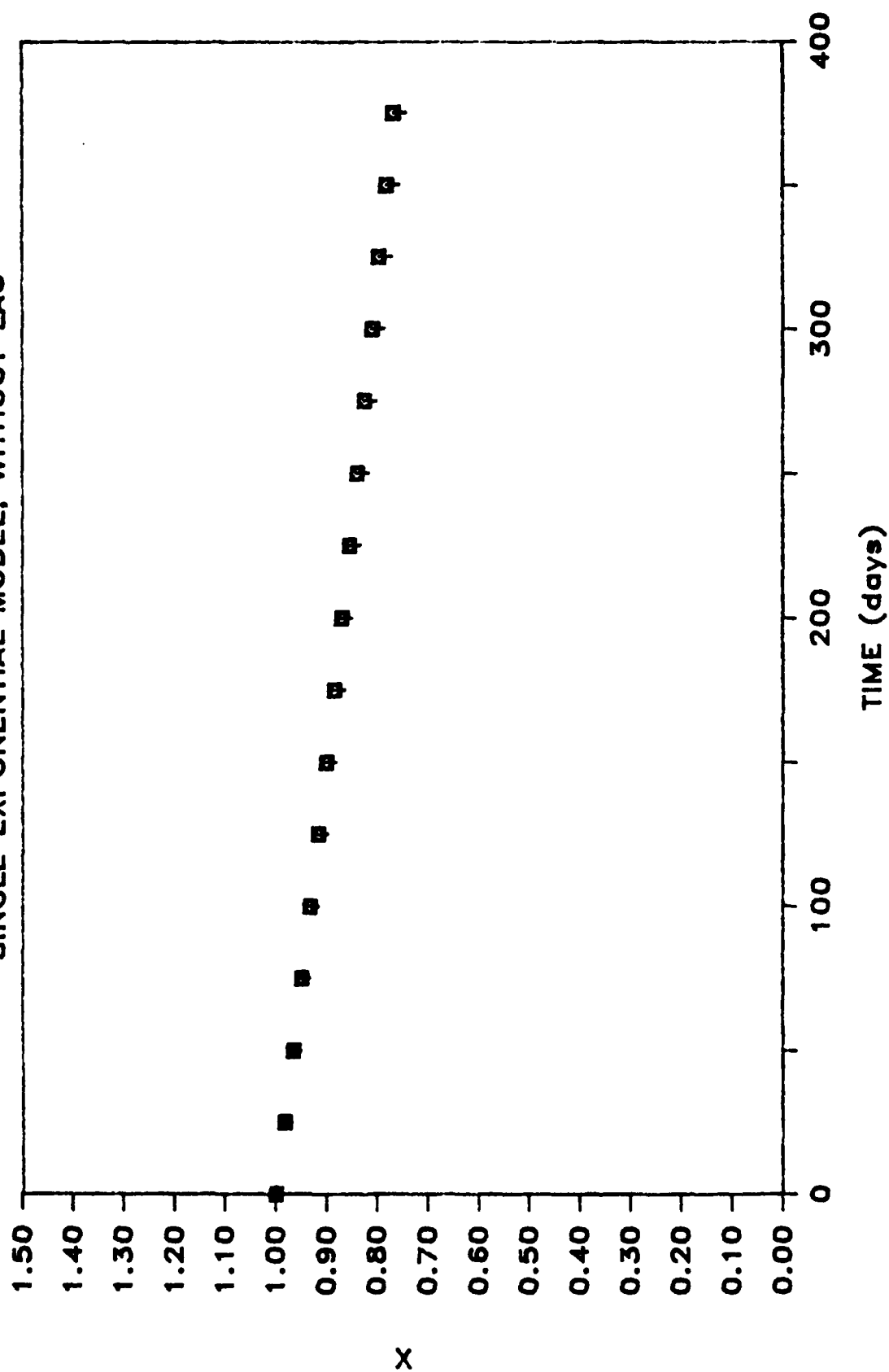
Figure 34. Forms of single exponential model best fitting overall mass loss by individual pine fascicles at the three study locations with lag period of 89 days.

BULK PINE LITTER, ALL THREE SITES SINGLE EXPONENTIAL MODEL, WITHOUT LAG



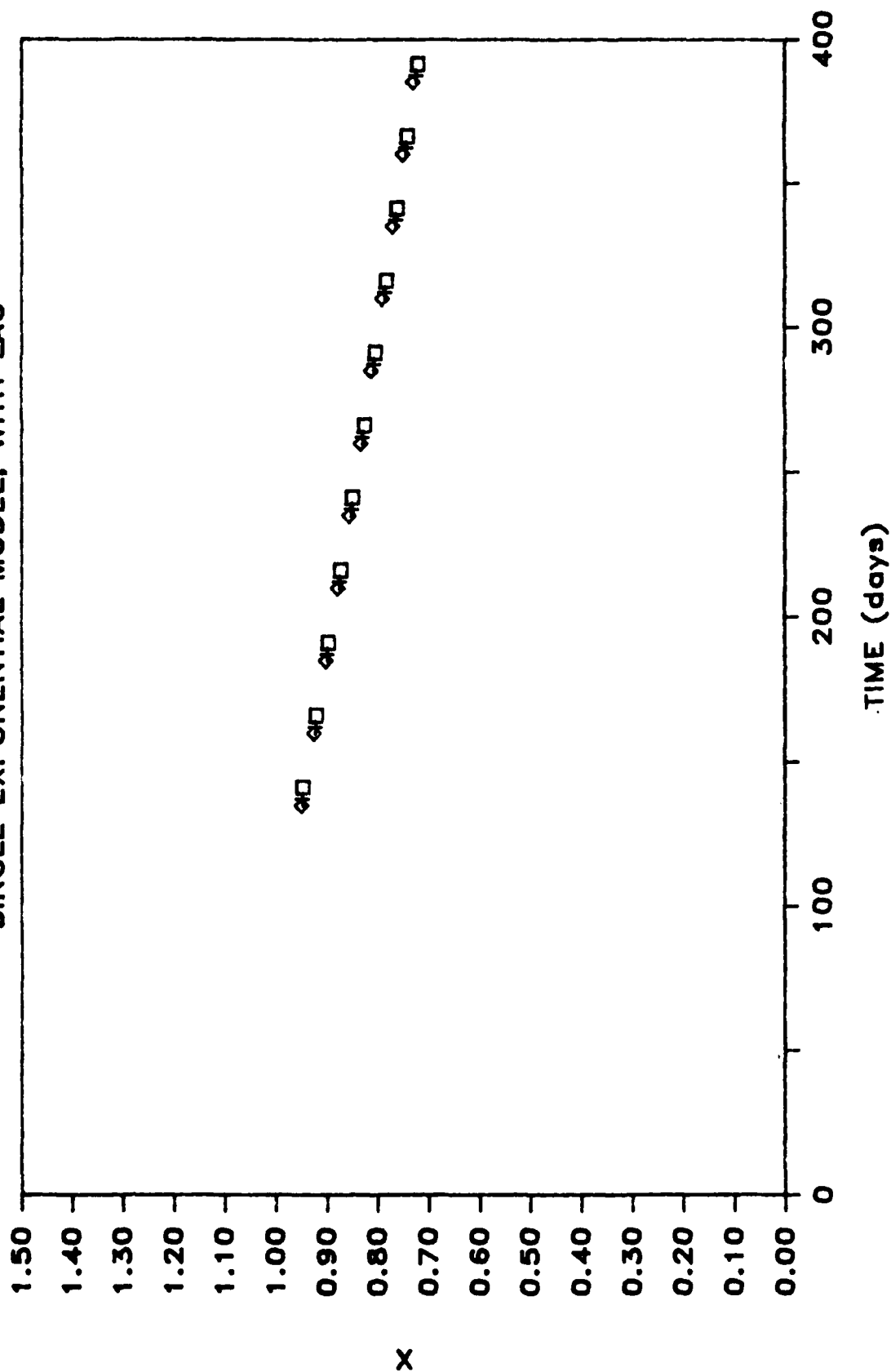
PINE FASCICLES, ALL THREE SITES

SINGLE EXPONENTIAL MODEL, WITHOUT LAG



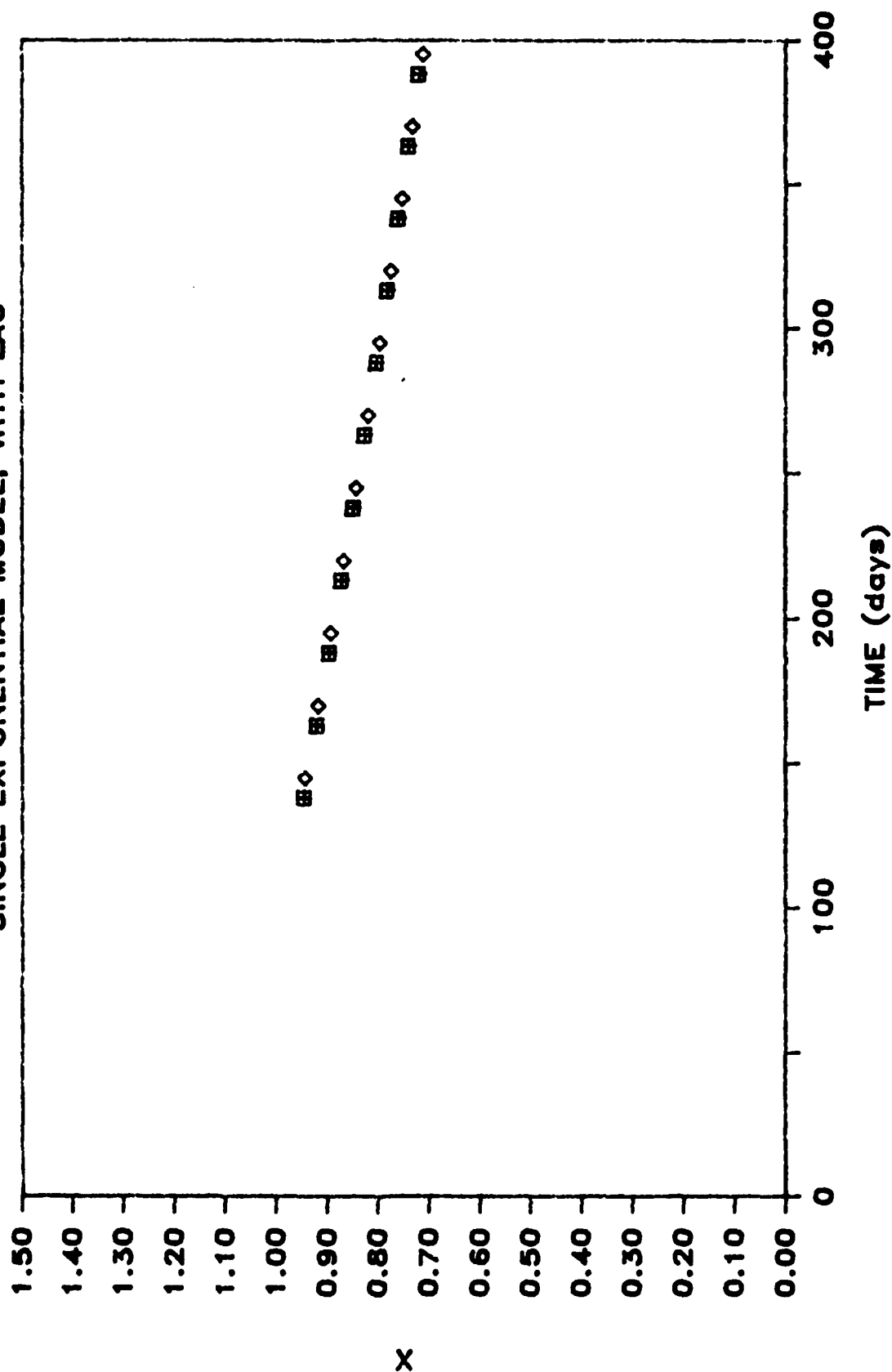
BULK PINE LITTER, ALL THREE SITES

SINGLE EXPONENTIAL MODEL, WITH LAG



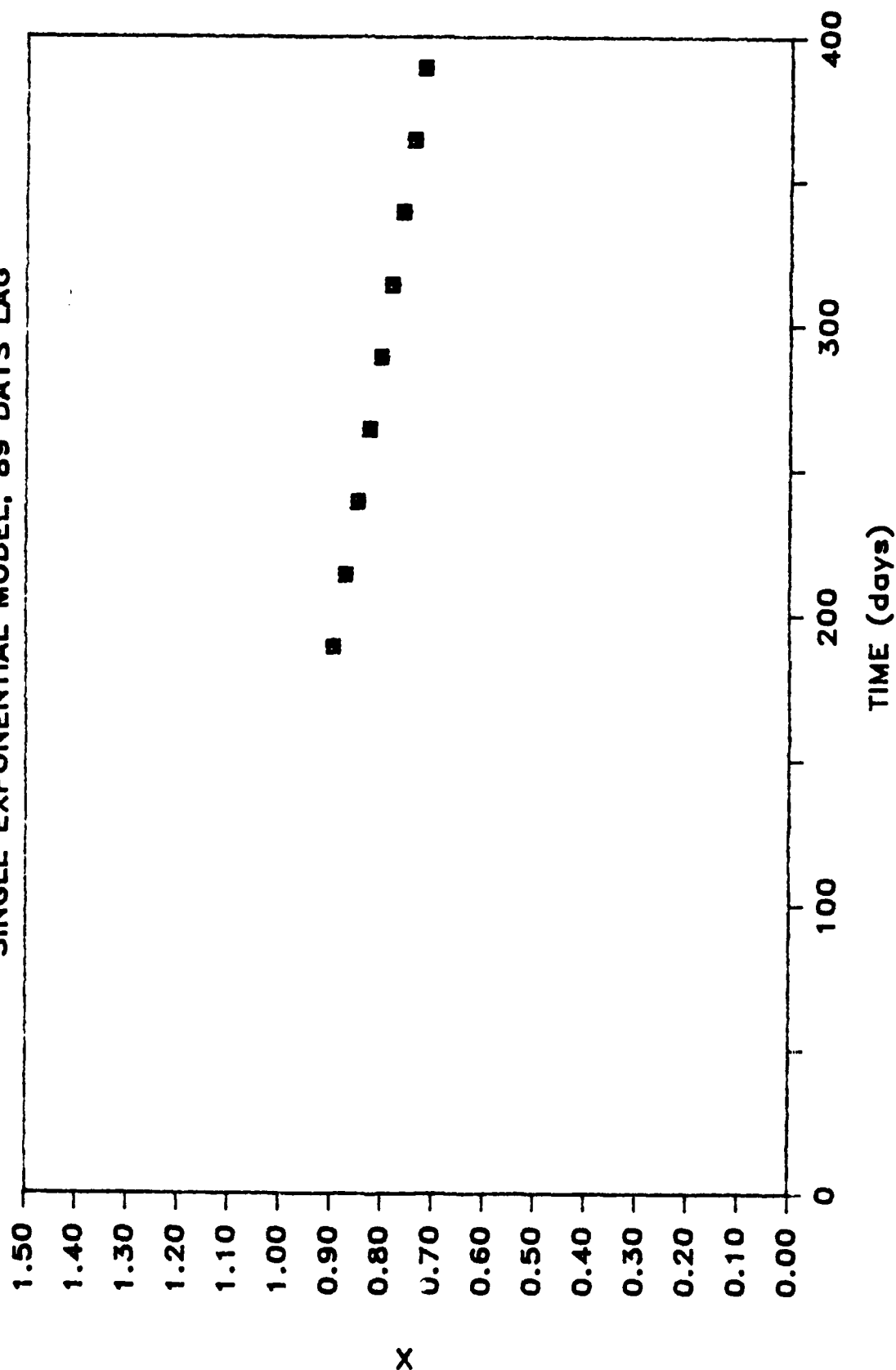
PINE FASCICLES, ALL THREE SITES

SINGLE EXPONENTIAL MODEL, WITH LAG



PINE FASCICLES, ALL THREE SITES

SINGLE EXPONENTIAL MODEL, 89 DAYS LAG



individual fascicle samples. Generally, moisture content was slightly greater in the bulk samples. Moisture content data has been tabulated and will be analyzed by analysis of variance in order to compare moisture content at recovery for bulk and tethered foliage samples within study locations and recovery dates.

On the other hand, phosphorus, potassium, calcium and magnesium behaved differently in the plantations than in the pole-stand (Tables 4-7, respectively). All four elements showed strong tendencies toward slower losses, or even recoveries (especially potassium), in the pole-stand compared to the two plantations. Nitrogen did not demonstrate this trend (Table 3). Differences between the plantations and pole-stands may be greater for the 1984-85 study than those which developed during the 1983-84 study, because the plantations were not cleared until June of 1984. The 1984-85 study plantation samples will spend the entire year in the plantations.

The possibility that a double exponential model might better fit the overall mass loss data was also explored using BMDPAR. The form of the model tested is:

$$X = Ae^{-k_1t} + (1-A)e^{-k_2t} ,$$

where A is the proportion of the initial mass relatively easily removed, (1-A) is the proportion representing more recalcitrant components (e.g., cellulose, lignin), k_1 and k_2 are rate constants for the decomposition of the two fractions of the initial mass, and t is the time elapsed since initiation of decomposition (Hunt 1977). A lag factor was also incorporated by substituting (t-1) for t in the equation above. Initial values for A, k_1 , and k_2 were set at 0.223, 0.0409, and 0.000971, based on data collected for oak leaves by Pinck et al. (1950). Attempts to fit double exponential models to weight loss data, with or without a lag period, all failed. In every case, the iterative process led to an extremely low value for the easily decomposed proportion of the initial mass. At best, the program appeared to approach a single exponential model in form.

Element 2: Rhizoplane and Rhizosphere Actinomycetes

Introduction

In the past year (1984), the emphasis of this element has moved more specifically to the enumeration and characterization of streptomycetes rather than the heterotrophic bacterial (HB) population in general. Individual non-streptomycete HB might be more susceptible to environmental changes and levels of nutrients than the streptomycetes (Goodfellow and Williams 1983), thus resulting in more variation between sites and seasons. Use of a restrictive medium such as starch casein agar (SCA) also means that only a certain portion of the HB population will be recovered from any sample. HB levels occurring on SCA were recorded for all of the 1984 samples, although little effort was placed on extensive characterization of HB types. Additional samples of mycorrhizae and soil were collected from red pine seedlings at the Toumey Nursery as well as from seedlings representative of the planting stock at the time of planting (June, 1984). This was done in order to assess possible changes in both streptomycete and mycorrhiza levels and types after the seedlings were planted at the test sites.

Several other changes have been made in the test protocol in order to more closely link this element with the nutrient cycling studies (Element 1, this report) and mycorrhiza studies (Elements 6 and 7, pages 75-95 of the Annual Report 1984 for the Herbaceous Plant Cover and Tree Studies project). For the first time, red pine litter samples were examined for streptomycete levels and types in order to begin determinations of how these organisms may be involved in litter decomposition (Orchard 1984). Two modifications were made to the analysis of washed root procedure in order to better understand and document the relationship between streptomycetes and mycorrhizae. The washed root samples consisted of mycorrhizal fine roots only, rather than a combination of mycorrhizal and non-mycorrhizal root sections (as in 1982-83); in addition, all the washed root samples were separated and analyzed according to mycorrhizal

type. A technique was developed for the recovery of streptomycetes associated specifically with individual mycorrhizal root tips (2 mm or less in length). The intent of adding this analysis was to provide data on even more specific streptomycete/mycorrhizae interactions to complement the washed root and rhizoplane soil studies, as this type of relationship is expected to be fairly stable with time.

Methods

All soil, washed root, and mycorrhizal root tip samples were collected and prepared by team researchers in the MTU Forestry Department and delivered to the Environmental Microbiology lab in the Department of Biological Sciences in sterile containers. These samples were usually processed within 24 hours of receipt. Samples were designated as to collection site, i.e., antenna, ground, or control. The types of samples analyzed during 1984 are listed in Table 9. Some litter samples were also examined during 1984.

Soil samples were first sieved through a 2 mm mesh screen in order to remove roots, rocks, etc. A one-gram (wet weight) portion of each soil sample was then weighed out and placed into 9.0 ml sterile dilution blanks (containing 0.01 M phosphate buffer, pH 7.2). Subsequent serial dilutions were made using the same type of sterile buffer. A larger portion of the sieved soil was transferred to a pre-weighed aluminum pan and weighed; this portion was then placed in a drying oven for soil dry weight determination.

Washed root samples were handled in a similar manner, except that additional precautions were taken to maintain aseptic conditions. Using flame-sterilized forceps, 0.1 g (wet weight) of washed roots was placed in 9.9 ml sterile phosphate buffer and homogenized in a flame-sterilized 30 ml blender. This mixture was then transferred to a sterile, screw-cap test tube; further dilutions were made in sterile phosphate buffer. Washed root dry weight determinations were made in the same manner as for the soil samples.

Table 9. Soil, root, and litter samples analyzed during 1984.

Date ^a	Sample Type ^b	Sampling Site			
		Nursery	Antenna	Ground	Control
April, 1984	Soil	2	-	-	-
	Root Tips	3	-	-	-
May, 1984	Litter	-	1	1	-
	Soil	-	9	9	9
June, 1984	Root Tips	-	1	3	3
	Soil	-	1	1	1
July, 1984	Washed Roots	-	1	1	1
	Root Tips	-	1	1	1
August, 1984	Litter	-	6	3	-
	Soil	-	2	4	2
September, 1984	Washed Roots	-	6	4	4
	Root Tips	-	3	4	4
October, 1984	Litter	-	5	1	-
	Soil	-	2	1	1
November, 1984	Washed Roots	-	3	3	3
	Root Tips	-	2	2	2
December, 1984	Soil	-	1	1	1
	Washed Roots	-	2	2	3
January, 1985	Root Tips	-	3	2	3

a April and June samples represent red pine seedlings and associated soil prior to planting.

b All samples were sorted by mycorrhiza morphology type prior to analyses.

The litter samples were processed for conducting plate counts similar to the soil samples. No further dry weight determinations were made as these had already been conducted in the Department of Forestry. A preliminary study showed that drying at 30°C did not affect recovery of streptomycetes from the litter.

As in the 1983 studies, all soil and root samples (after preparation and appropriate serial dilution) were spread-plated onto starch casein agar (SCA) in 100 mm petri dishes. Cycloheximide (50 mg/l) and nystatin (50 mg/l) were added to the SCA to prevent fungal growth (Andrews and Kennerly 1979, Goodfellow and Dawson 1978). Three to four dilutions (in duplicate) were spread-plated per sample. All plates were incubated at 20°C. Total numbers of heterotrophic bacterial colonies and streptomycete colonies were determined after 7 and 14 days incubation.

After 14 days incubation, strain diversity estimates were made for streptomycete colonies growing on SCA. All colonies with the same characteristics were considered to represent one type or strain. At least one colony per streptomycete type was isolated in pure culture for further study. Using the format of Shirling and Gottlieb (1966), the streptomycete cultures were characterized for melanin production, color of aerial mycelia, production and color of reverse and soluble pigments, sporophore structure, and carbohydrate utilization. Additional tests were conducted to evaluate calcium oxalate (Jayasuriya 1955, Knutson et al. 1980), cellulose, and lignocellulose (Sutherland 1985) degradation. The streptomycete types found in the 1984 samples were compared to those observed in similar samples from 1983 to determine if some of the same types were present.

Enrichment techniques were developed specifically for isolating streptomycetes from short (2 mm or less) portions of root tips representing specific mycorrhiza morphology types. In preliminary experiments, starch casein broth (SCB) with cycloheximide and nystatin was supplemented with sodium propionate (SCB-SP, 4 g/l), rose bengal (SCB-RB, 0.035 g/l) or

aureomycin (SCB-A, 0.00005 g/l) (Kutzner 1981). These agents were added to reduce bacterial but not streptomycete growth. Each medium was first tested with pure streptomycete and bacterial cultures to determine its effect on their growth compared to SCB. All three media were inhibitory to bacteria, but SCB-SP was least inhibitory to streptomycetes (i.e., little or no inhibition). All further root tip enrichments were therefore conducted using SCB-SP.

Root tip enrichments were first conducted by aseptically transferring one mycorrhizal root tip portion to a 25-ml flask containing 5 ml SCB-SP. Up to 20 root tips were tested per type. The flasks were placed on a floor model rotary shaker at moderate speed for up to 96 hrs at 20-22°C. Broth was streaked onto SCA at 0, 24, 48, and 96 hrs. After incubation at 20°C, the plates were examined for streptomycete colonies. All streptomycete colonies were transferred to fresh SCA to obtain pure cultures and were saved for characterization studies.

Because of excessive bacterial growth with some of these enrichments after 24 hrs, the flasks with SCB-SP and a root tip in later studies were heated to 55°C for 6 min before shaking in order to reduce bacterial levels (Orchard 1984). In studies with pure cultures of streptomycetes and root samples, this heat treatment did not affect detection of streptomycetes, but reduced levels of bacteria and some fungi which would otherwise have outgrown the streptomycetes in the enrichment flasks. Some type of preheat treatment is commonly used when working with natural populations of streptomycetes, as their spores in particular can withstand these temperatures (as reviewed in Kutzner 1981; Williams *et al.* 1972).

Data for streptomycete levels were transformed to log₁₀ (Orchard 1984) and evaluated statistically using a block-treatment design analysis of variance to compare sampling dates, sampling sites, and sample types at the $\alpha = 0.05$ significance level (Zar 1984), with sampling sites as "blocks" and sampling dates/types as "treatments". Where the analysis showed significant differences among treatments, Duncan's new

multiple range test was employed to conduct multiple comparisons in order to determine which treatments were significantly different (Steel and Torrie 1980). No statistical analyses were conducted for the HB data.

Description of Progress

As was noted previously, only red pine root and associated soil samples were analysed during the 1984 sampling season, and, in order to collect background data on streptomycete numbers and types before as well as after planting of nursery red pine seedlings on the various plantation sites, washed root, root tip, and soil samples were collected from red pine seedlings in place at the Toumey Nursery and also just before planting. Soil samples were collected at the antenna, ground, and control sites before the red pine seedlings were planted. Additional soil, washed root, and root tip samples were then collected on a monthly basis through October from each of the antenna, ground, and control plantation sites in order to begin to follow any changes in streptomycete levels and types on the seedling roots and in the soil.

Streptomycete and HB levels from nursery/rhizoplane soil and mycorrhizal washed roots are presented in Table 10. Too few samples were collected for statistical analysis, particularly for the seedlings later planted at specific sites. As was found in the previous years' studies, the nursery environment selects for relatively few types of streptomycetes as compared to the number of types found with naturally occurring red pine in the field.

Data for all antenna, ground and control site soil samples and for washed mycorrhizal root samples collected after the planting of the nursery red pine seedlings are presented in Table 11. All soil samples were collected from or near mycorrhizal roots in the O horizon. In comparing streptomycete levels in soil collected before (22 June 1984) and after the red pine seedlings were planted (24 July 1984 on), the only significant difference between sampling dates and sites was for those samples collected before the seedlings were planted. This difference may

Table 10. Heterotrophic bacteria and streptomycetes associated with washed red pine seedling roots and rhizosphere soil at the nursery and before planting.

Sample Type	Sampling Date	Sample Site	Viable Counts ^a			Number of Streptomycete Types
			Heterotrophic Bacteria	Streptomycetes		
Soil	9 April 1984	Nursery	$5.9 \times 10^7 \pm 2.2 \times 10^{7e}$	$4.8 \times 10^5 \pm 1.4 \times 10^{5e}$		4 ± 1^e
	22 June 1984	Ground ^c	$2.4 \times 10^8 \pm 2.7 \times 10^{8d}$	$4.5 \times 10^6 \pm 6.6 \times 10^{5d}$		3 ± 1^d
	22 June 1984	Control ^c	$2.2 \times 10^7 \pm 2.8 \times 10^{6e}$	$8.9 \times 10^5 \pm 1.7 \times 10^{5e}$		2 ± 0^e
Washed Roots	9 April 1984	Nursery	1.5×10^7	2.3×10^6		3
	22 June 1984	Antenna ^c	$1.8 \times 10^7 \pm 2.1 \times 10^{7e}$	$4.5 \times 10^4 \pm 4.2 \times 10^{4e}$		4 ± 2^e
	22 June 1984	Ground ^c	$5.1 \times 10^6 \pm 2.4 \times 10^{6e}$	$1.5 \times 10^6 \pm 4.9 \times 10^{5e}$		2 ± 0^e
	22 June 1984	Control ^c	3.5×10^6	6.7×10^5		5

^a Reported per g dry weight of sample.

^b Includes numbers of streptomycetes.

^c From red pine seedlings just before planting at this site.

^d Mean \pm S.D. for 3 samples.

^e Mean \pm S.D. for 2 samples.

Table 11. Heterotrophic bacteria and streptomycetes associated with washed red pine seedling roots and rhizosphere soil from plantation sites.

Sampling Date	Sample Site	Soil			Washed Roots		
		Heterotrophic Bacteria ^b	Streptomycetes	Number of Streptomycete Types	Heterotrophic Bacteria ^b	Streptomycetes ^c	Number of Streptomycete Types
22 June 1984	Antenna	$9.6 \times 10^7 \pm 7.4 \times 10^7$ ^d	$7.0 \times 10^6 \pm 3.4 \times 10^6$ ^d	9 ± 2 ^d	---	---	---
	Ground	$2.1 \times 10^8 \pm 2.4 \times 10^8$ ^d	$3.2 \times 10^6 \pm 2.5 \times 10^6$ ^d	9 ± 5 ^d	---	---	---
	Control	$5.1 \times 10^8 \pm 3.9 \times 10^8$ ^d	$7.1 \times 10^6 \pm 6.7 \times 10^6$ ^d	3 ± 2 ^d	---	---	---
24 July 1984	Antenna	6.7×10^5	1.6×10^5	11	2.4×10^6	7.8×10^5	4
	Ground	1.3×10^6	1.0×10^5	12	1.5×10^6	3.8×10^5	6
	Control	5.6×10^5	4.7×10^5	9	7.2×10^6	1.7×10^6	5
22 August 1984	Antenna	$1.0 \times 10^6 \pm 3.2 \times 10^5$ ^e	$7.5 \times 10^5 \pm 6.3 \times 10^5$ ^e	10 ± 3 ^e	$5.5 \times 10^7 \pm 3.6 \times 10^7$ ^e	$7.7 \times 10^5 \pm 8.3 \times 10^5$ ^e	5 ± 2 ^e
	Ground	$4.9 \times 10^6 \pm 1.8 \times 10^6$ ^e	$9.9 \times 10^5 \pm 6.4 \times 10^5$ ^e	6 ± 2 ^e	$1.5 \times 10^8 \pm 1.0 \times 10^8$ ^e	$9.9 \times 10^5 \pm 1.9 \times 10^6$ ^e	5 ± 1 ^e
	Control	$1.1 \times 10^6 \pm 5.2 \times 10^5$ ^e	$7.7 \times 10^5 \pm 2.7 \times 10^5$ ^e	10 ± 1 ^e	$1.2 \times 10^8 \pm 1.7 \times 10^8$ ^e	$8.3 \times 10^5 \pm 8.1 \times 10^5$ ^e	5 ± 2 ^e
23 September 1984	Antenna	3.4×10^6	2.3×10^6	6	f	f	f
	Ground	5.9×10^5	6.5×10^5	7	f	f	f
	Control	2.3×10^6	3.4×10^5	7	f	f	f
29 October 1984	Antenna	5.3×10^5	3.9×10^5	7	$6.9 \times 10^5 \pm 2.8 \times 10^5$ ^e	$3.6 \times 10^5 \pm 3.1 \times 10^5$ ^e	5 ± 1 ^e
	Ground	8.4×10^5	9.2×10^4	5	$5.1 \times 10^5 \pm 2.0 \times 10^5$ ^e	$7.8 \times 10^4 \pm 2.3 \times 10^4$ ^e	3 ± 1 ^e
	Control	7.3×10^5	2.5×10^5	5	$1.1 \times 10^6 \pm 3.7 \times 10^5$ ^e	$1.7 \times 10^5 \pm 8.8 \times 10^4$ ^e	2 ± 1 ^e

a Reported per g dry weight.

b Includes numbers of streptomycetes.

c Composite of mycorrhizal rootlet morphology types present.

d Mean \pm S.D. for 3 samples.e Mean \pm S.D. for 2 samples.

f N.A. = data not available due to contamination.

be partially due to the environmental influences of clearcutting the pre-existing hardwood cover in preparation for planting. It is also possible that soil streptomycete levels from July on may simply reflect the influence of the pine seedlings on their rhizosphere. There was no statistically significant difference in washed root streptomycete levels between the three sites and dates (July - October, 1984). As was found previously, fewer streptomycete types were found with the washed roots than with the soil. It is interesting to note that, unlike most of the samples from the 1982-83 studies, there was no significant difference between the washed root streptomycete levels and the soil streptomycete levels. This may possibly be related to the difference in type of washed roots between the past and present studies, i.e., only mycorrhizal roots were examined during the present study, and may reflect an "enrichment" situation for streptomycetes created by the mycorrhizae.

Data for streptomycete levels and types and HB levels are presented in Table 12 for each mycorrhizal morphology type found with the red pine seedlings in the nursery, just before planting, and after planting at the antenna, ground, and control sites. The presence and abundance of specific mycorrhizal morphology types is documented in Table 31, page 91 of the Annual Report 1984 for the Herbaceous Plant Cover and Tree Studies project. It appears that there were some differences in streptomycete types associated with the different mycorrhizal types encountered and that additional changes may have occurred since planting of the seedlings at the various sites. No significant difference was found between levels of streptomycetes detected with the type 3 mycorrhizal morphology washed roots at the control, ground and antenna sites on the various dates on which this mycorrhiza type was detected. There was also no difference between streptomycetes associated with mycorrhizal morphology types 2 and 3. Too few data points were available for analysis for the two other mycorrhizal morphology types.

The enrichment technique data obtained with small mycorrhizal root tip sections are presented in Table 13. As was

Table 12. Microscopic bacteria and microspores associated with specific morphological bacterial morphologies.

Sampling Date	Bacterial Type 1				Bacterial Type 2				Bacterial Type 3				Bacterial Type 4			
	Sample Size	Microscopic Bacteria	Microspores	Number of Microspores	Microscopic Bacteria	Microspores	Number of Microspores	Microscopic Bacteria	Microscopic Bacteria	Microspores	Number of Microspores	Microscopic Bacteria	Microscopic Bacteria	Microspores	Number of Microspores	Microscopic Bacteria
9 April 1960	100	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³
22 June 1960	100	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³
20 July 1960	100	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³
22 August 1960	100	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³
29 September 1960	100	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³	1.0 x 10 ³	1.0 x 10 ³	1	1.0 x 10 ³

a. Reported are a dry weight of mixed mass.
 b. Number of bacteria of microspores.
 c. Number from primary isolation prior to plating.
 d. This percentage type was not found at this site on this date.
 e. Data - 1.0 x 10³ sample.

Table 13. Streptomycete enrichments from mycorrhizal root tips.

Sampling Date	Sample Type	Mycorrhizal Rootlet Morphology Type	Streptomycete Type
4 April 1984	Planting Stock ^a	2	2
		5	2
22 June 1984	Planting Stock ^b	3	1
	Planting Stock ^c	3	1
24 June 1984	Antenna	3	3
	Ground	3	1
22 August 1984	Antenna	2	0
		3	1
		6	1
	Ground	2	0
		3	0
	Control	2	1
		3	1
23 September 1984	Antenna	2	0
		3	1
	Ground	2	1
		3	0
	Control	2	0
		3	1
29 October 1984	Antenna	2	0
		3	1
		5	0
	Ground	2	1
		3	1
	Control	2	0
		3	1
		6	1

^a Sampled in nursery.

^b Prior to planting at ground site.

^c Prior to planting at control site.

mentioned in the Methods section, this technique was changed somewhat during the course of the 1984 sampling season; refinements are still being made in order to reduce bacterial and fungal contamination, promote streptomycete growth, and increase the number of samples/tips that can be analysed at any one time. As was indicated previously, this technique is being used for the specific purpose of detecting streptomycetes and not other soil microorganisms by using the same nutrients as in the plate count technique plus a preheat treatment. These data do indicate that specific streptomycetes are associated with individual mycorrhizal tips, although the number of types is lower than with the washed roots (Tables 10 and 12). This is possibly due to the very small mass being tested in the enrichments and some of the problems listed above relating to contamination by other microbes, etc. The streptomycetes found in the enrichments can be compared to those found with the washed roots and may be used in later studies on specific streptomycete/mycorrhiza interactions.

The levels of streptomycetes and HB associated with the litter samples from all sites varied greatly (Table 14). In fact, significantly higher levels of streptomycetes were found with litter from the ground site plantation vs the antenna site plantation. All streptomycete levels were lower than those found in the underlying soil (Table 11). Orchard (1984) reported similar results when testing ryegrass litter and underlying soil.

It is apparent that many of the same streptomycetes types were associated with the soil and washed root samples from 1984 as were found with comparable samples from 1983. The streptomycete isolates characterized represent 10 or more of the 23 types characterized during the previous study, i.e., types 2, 7 - 10, 14, 15, 18, 20, and 21. (An updated table listing the streptomycetes previously characterized is presented in Appendix A).

The predominant streptomycete type found with red pine nursery seedling washed roots in 1983 (type 2) was again found with the same type of samples in 1984. This streptomycete type

Table 14. Heterotrophic bacteria and streptomycetes associated with red pine leaf litter.

Sampling Date	Sampling Site	Viable Counts ^a		Number of Streptomycete Types
		Heterotrophic Bacteria ^b	Streptomycetes	
11 May, 1984	Antenna - Plantation	1.5×10^4	2.0×10^2	2
	Ground - Plantation	7.3×10^4	1.5×10^4	2
1 July, 1984	Antenna - Plantation	$6.4 \times 10^5 \pm 5.0 \times 10^5$ c	$2.2 \times 10^2 \pm 1.5 \times 10^2$ c	2_1^+ c
	Antenna - Pole Stand	$3.2 \times 10^5 \pm 9.2 \times 10^4$ c	$1.1 \times 10^4 \pm 1.0 \times 10^2$ c	4_2^+ c
	Ground - Plantation	$1.35 \times 10^6 \pm 1.4 \times 10^6$ c	$2.2 \times 10^4 \pm 1.1 \times 10^4$ c	4_2^+ c
	Antenna - Plantation	8.5×10^5	1.0×10^4	1
1 August, 1984	Antenna - Pole Stand	$1.7 \times 10^6 \pm 7.3 \times 10^6$ c	$3.3 \times 10^3 \pm 3.3 \times 10^3$ c	2_1^+ c
	Ground - Plantation	6.9×10^5	1.2×10^4	2

a Reported per g dry weight.

b Includes numbers of streptomycetes.

c Mean \pm S.D. for 2 samples.

apparently was not found in any of the soil samples from the control, antenna, or ground sites. Based on isolates characterized to date, streptomycete type 2 was present on the red pine seedling washed roots at least through August, 1984.

The streptomycete types found associated with red pine foliar litter were also found in the underlying soil; however, as noted earlier, fewer types were found with the litter than with the soil samples.

Based on the data collected in 1984, no statistically significant differences were detected in populations of streptomycetes associated with mycorrhizal roots between sites. The only difference in the soil samples occurred not between sites but between sampling dates (due to one date). This is certainly partly due to the large natural variation within sites and samples and is typical for the complex soil/root system. Use of selective media, however, may result in less variation with streptomycete counts. Nevertheless, in light of the need for more statistically precise data, the following changes will be made in the program of study during 1985.

Work in 1985 will concentrate only on streptomycetes and will be focused on mycorrhizal fine root samples. In order to increase the statistical power of this study, samples for these analyses will again be collected in conjunction with Mycorrhiza Development and Root Growth studies (Element 8 of the Herbaceous Plant Cover and Tree Studies project) and will be analyzed in triplicate from the control, ground and antenna sites on a monthly basis from May through October, 1985. Additional data on rhizoplane soil moisture and pH will also be collected to determine if these variables might influence streptomycete levels and types, as well as providing supplementary information for the mycorrhiza studies. There will be reduced emphasis on testing red pine litter samples as the 1984 study indicated that streptomycete levels in litter vary between sites and are often at such low levels that the streptomycetes may not have a significant role in litter decomposition (although about 50 per cent of the litter streptomycetes were found to degrade

cellulose). Soil samples will be deleted from the test program. No additional samples will be collected from nursery red pine seedlings.

The enrichment technique to detect streptomycetes associated with individual typed mycorrhizae will also be continued during 1985. As several laboratory studies have shown that a short period of heat treatment before incubation does not affect the streptomycete portion of the microbial population but does reduce HB levels, this preheat treatment will also be used as part of the enrichment study. From 20 to 30 individual mycorrhizal root tips per mycorrhiza type will be tested per sample in order to provide data for statistical analysis on incidence of specific streptomycete types occurring in association with specific mycorrhiza types at each sampling site on each sampling date.

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APPENDIX A
Description of Streptomycete Types

ix A. Description of Streptomyces Types^A

Fungus No.	Color of Aerial Mycelium	Production of ^B			Sporophore Shape	Utilization of										
		Melanin	Reverse Pigment	Soluble Pigment		Oxalate	Cellulose	Ligno-Cellulose	Arabinose	Sucrose	Xylose	Inositol	Mannitol	Fructose	Rhamnose	Raffinose
red	red	-	orange	orange	S	-	-	-	+	+	+	+	+	+	+	+
red	red	-	red	-	S	+	-	-	+	-	-	+	+	+	+	+
grey	grey	-	black	-	S	+	-	-	-	-	-	-	-	-	-	-
grey	grey	-	orange	-	S	-	-	-	+	+	+	+	+	+	+	+
white	white	-	-	-	S	+	-	-	+	+	+	+	+	+	+	+
grey	grey	-	yellow-brown	yellow-brown	F	+	+	+	NT	-	-	-	NT	NT	+	NT
white	white	-	yellow-brown	-	F	-	-	-	+	-	-	-	NT	+	+	NT
grey	grey	-	yellow-brown	-	F	+	+	+	+	+	+	+	+	+	+	NT
lt. grey	lt. grey	+	yellow-brown-red	-	F	+	+	+	+	+	+	+	+	+	+	NT
white	white	-	brown	-	F	+	+	+	+	+	+	+	+	+	+	NT
grey	grey	-	yellow-brown	yellow	S	-	-	-	+	+	+	+	+	+	+	NT
lt. grey	lt. grey	-	brown	-	S	-	-	-	+	+	+	+	+	+	+	NT
white	white	-	yellow-brown	yellow	S	-	-	-	+	+	+	+	+	+	+	NT
grey	grey	-	yellow-brown	-	F	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
grey	grey	-	yellow-brown	-	F	+	-	-	+	+	+	+	+	+	+	+
white	white	+	yellow-brown	-	S	-	-	-	+	+	+	+	+	+	+	+
white	white	+	-	-	F	-	-	-	+	+	+	+	+	+	+	+
dk. grey	dk. grey	+	brown	-	S	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
yellow	yellow	-	-	-	P	+	+	+	+	+	+	+	+	+	+	+
dk. grey	dk. grey	-	green	green	S	+	-	-	+	+	+	+	+	+	+	+
white	white	-	yellow-brown	yellow	S	+	+	-	+	+	+	+	+	+	+	+
lt. grey	lt. grey	-	-	yellow	F	+	+	-	+	+	+	+	+	+	+	+
dk. grey	dk. grey	-	dk. brown	yellow-brown	S	-	+	+	+	+	+	+	+	+	+	+

ing the general format of Shirling and Gottlieb (1968), with pigment/morphology descriptions made on yeast
ract/malt extract, glycerol-asparagine, and/or peptone-yeast extract-iron agar.

or, if produced.

simple; F, flexuous; P, Spiral.

ared calcium oxalate agar.

- Not tested.

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Subcontract #E06549-84-C-009

"ELF Communications System Ecological Monitoring Program"

**The Effects of Exposing the Slime Mold Physarum polycephalum
to Electromagnetic Fields**

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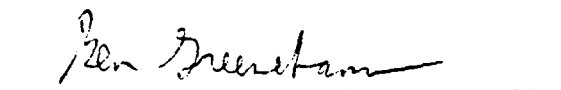
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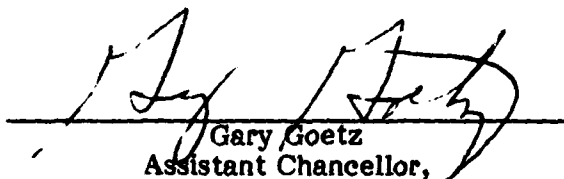
January, 1985



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GLOSSARY - ACRONYMS

Respiration:	A measurement of the rate of oxygen utilization.
Antenna ground:	A conducting connection between the transmitting antenna and the earth.
Axenic culture:	Growth of a single organism (slime mold) in the absence of contaminating organisms such as bacteria, fungi, etc.
Plasmodium:	A mass of protoplasm visible to the eye containing numerous nuclei; the entire structure is delimited by a plasma membrane. In the laboratory it is usually maintained on a solid substrate such as agar or filter paper.
Micro-plasmodia:	Plasmodia maintained in submerged shake flasks.
Shake flask cultures:	A method of maintaining plasmodia in a liquid nutrient medium. The flask is continuously shaken to provide oxygen to the culture.
Cell cycle:	The number of hours between successive divisions of a cell; in this experiment it is the number of hours required for division of the nucleus.
W.T.F.	Wisconsin Testing Facility.
ELF	Extremely low frequency fields.
I.I.T.R.I.	Illinois Institute of Technology Research Institute.

TABLE OF CONTENTS

	PAGE
INTRODUCTION	1
ELEMENT 1. Selection of Control and Experimental Sites	2
ELEMENTS 2 and 7. Protocols for Field Exposure and Maintenance of <u>Physarum</u>	
2. Field Exposure	2
7. Culture Maintenance	3
ELEMENTS 3 and 4. Laboratory Exposure of <u>Physarum</u>	4
Results and Discussion of Laboratory Data	5
EFFECTS OF FIELD EXPOSURE AT THE WISCONSIN TEST FACILITY	
1. Mitosis	6
2. Respiration	7
OTHER EXPERIMENTS	8
SUMMARY OF DATA	9
DATA	10
APPENDIX I. Location of Sites and Field Intensity	
Measurements	45
APPENDIX II. Daily Record of Temperatures at Field Sites	53
APPENDIX III. Statistical Analysis	57
APPENDIX IV. Method for Computation of Paired T-Test	61
REFERENCES	65

ABSTRACT

We have previously shown that continuous laboratory exposure of the slime mold Physarum polycephalum to extremely low frequency weak electromagnetic fields (ELF-EMF) similar to those generated by the Navy's ELF communication antenna can depress the rate of respiration and lengthen the mitotic cell cycle (Goodman et al. 1976, 1979, Greenebaum et al. 1982). We now seek to determine whether exposing Physarum to the actual field environment around the Wisconsin Test Facilities (WTF) communications antenna will induce an altered physiological state.

To answer this question, a research program comprising both a laboratory and field component has been developed. Since all of our earlier experiments involved only continuous ELF-EMF exposure, it was necessary first to determine whether intermittent ELF-EMF exposure was capable of altering the cell's physiology. This was important because the field experiments conducted during both 1983 and 1984 would be performed with the antenna operating in an intermittent mode. Thus, if the tightly controlled laboratory experiments indicated that intermittent exposure had no effect, there would be little reason to suspect that exposure to the fields around the antenna (in an uncontrolled environment) would induce significant physiological changes.

Experiments were performed in the laboratory during 1983 in which the mold was exposed to a continuous 76 Hz, sinusoidal field (CW) 1.0 G., 1.0 V/m for 16 hours per day, 5 days/week. This exposure regimen lengthened the mitotic cell cycle and increased the respiration rate (Dec. 1983 report, Goodman et al. 1984). The lengthened mitotic cycle agreed with the results previously reported. However, the enhanced respiration rate was the opposite of our findings with continuous exposure. These results suggest that in addition to field intensity and waveform, the exposure period is also an important parameter in elucidating the bio-effects of weak fields.

INTRODUCTION

We have previously shown that continuous laboratory exposure of the slime mold Physarum polycephalum to extremely low frequency weak electromagnetic fields (ELF-EMF) similar to those generated by the Navy's ELF communication antenna can depress the rate of respiration and lengthen the mitotic cell cycle (Goodman et al. 1976, 1979, Greenebaum et al. 1982). We now seek to determine whether exposing Physarum to the actual field environment around the Wisconsin Test Facilities (WTF) communications antenna will induce an altered physiological state.

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Having established that intermittent laboratory exposure could induce cell effects, our efforts during 1984 were directed to determining whether exposing Physarum to the ELF-EMF generated by the communications antenna would induce

similar perturbations in both mitosis and respiration. To provide the reader with a better perspective of the program, we have included a brief review of some tasks that were completed and described in our 1983 report.

ELEMENT 1: SELECTION OF CONTROL AND EXPERIMENTAL SITES

Four sites (1 control, 3 experimental) were used in the field studies during 1984. The first exposure site is located parallel to the west ground (G-3, see map, Appendix 1), the second (A-2) is a new site located about 3 miles from the ground site below the antenna (see Appendix 1 for specific locations), and the third site (A-1) is located above a buried section of the antenna. The control site (C-1) was located about 20 miles east of the ground site. Although the A-1 site does not meet the 60 Hz magnetic field exposure criterion (A-1/C-1 should be about 1.0), experiments were continued to provide a frame of reference and continuity from the previous year.

Field measurements at all sites were made both by IITRI at the beginning of the season, and by us at various times during the summer; these data are summarized in Appendix 1. In general, measurements were made when the antenna was at peak power; field intensity measurements at C-1, A-1, and G-3 matched well with measurements made the previous years.

One problem detected by IITRI was the fact that the electric field intensities in the soil exceeded those in the culture chamber. This difference is the result of a mismatch of the conductivities in the growth chamber system and the earth. To address this problem the distance between the collector electrodes will be increased and a chamber assembly will be constructed that will allow one to more closely match the conductivity of the chambers and the soil, the new chambers will be used during the 1985 season.

ELEMENTS 2 AND 7: PROTOCOLS FOR FIELD EXPOSURE & MAINTENANCE OF PHYSARUM.

FIELD EXPOSURE SYSTEM: Plamodia were placed in the vicinity of the WTF antenna on 5/23/84 and were maintained in the field until 10/27/84. Cultures were grown

in autoclavable polyethylene chambers (7" x 4" x 2-1/4") with a tight fitting top; two carbon electrodes have been placed 6" apart and about 1/4" from the bottom of each chamber. Two or three growth chambers were placed inside the outer protective chamber (10" x 10" x 12"); a tight fitting lid provided a water-proof environment for the cultures. A 1/2" U-shaped vent pipe was attached to the lid of the outer chamber to facilitate gas exchange. The protective chambers with the growth chambers inside) were placed in a hole approximately 2" x 20" x 20"; 8" square copper collecting plates were buried at each end of the hole (in line with the predominant electric field). Electric fields are brought to the growth boxes by wire leads that run from the collecting plates to a plug on the outer wall of the protective chamber; multiple leads run from the plug to the carbon electrodes of the growth chambers. To protect the system from direct sunlight, foraging animals, etc., each hole was covered with a plywood board and overlain with a 2" layer of dirt. Each site contained at least two protective chambers with their associated cultures.

To continuously monitor temperature at each site, 7 day, temperature recorders (Bacharach-Tempscribes) were placed in one of the protective chambers at each site (except A-1). A temperature summary shows that the C-1 site tracked slightly warmer (1-2° F) throughout the season (see Appendix 2). In August, the recorder at the ground site malfunctioned; a replacement could not be secured in time to allow for meaningful monitoring during the remaining part of the season. As one would expect there is a general trend toward an increased ambient temperature that peaks in August, and then declines.

CULTURE MAINTENANCE: The field exposed cultures were maintained in an axenic state on a medium consisting of 50% growth medium (Daniel and Baldwin, 1964) 1% (w/v) sterile, rolled oats (Quaker), and 3% agar. All media preparation and sterilizations were performed at UW-Parkside; growth containers were placed in plastic bags and transported to the site.

To transfer the cultures in the field the following protocols were followed: (1) The outer chambers were disconnected from the collector plates and brought to the mobile lab where the outside of the container was thoroughly washed to remove mud and dirt. (2) The growth chambers were removed and the outer surfaces cleaned using a disposable wipe saturated with the disinfectant-Zorbicide. (3) The growth chambers were placed in a Baker, laminar-flow hood and the plasmodia subcultured to fresh growth medium; samples were simultaneously taken for experiments on mitosis and QO_2 by placing plasmodia on Petri dishes (containing the same growth medium), or in a bottle containing liquid growth media. These protocols generally proved effective, with little contamination encountered at either the control, A-1 or A-2 sites. Unfortunately, we did encounter contamination at the ground site mid-way through the program (week 9) all cultures at this site were lost and had to be restarted.

Plasmodia exposed at the WTF were placed in liquid nutrient media, returned to Parkside and placed in an incubator ($26^{\circ}C$) to which neither electric nor magnetic fields were applied. Within 24 hours of returning to Parkside, plasmodia were transferred to fresh medium and maintained as shake flask cultures in 125 ml Erhlenmeyer flasks until growth was adequate to perform experiments on mitosis and QO_2 (using an oxygen electrode). In general, the time between removal from the test site and performance of the test ranged between 5 to 7 days depending on the rapidity with which cultures readapted to liquid growth conditions. Plasmodia that had been placed on Petri dishes containing nutrient agar were used to measure oxygen consumption with an S-3A Oxygen Analyzer (Applied Electrochemistry).

ELEMENTS 3 AND 4: LABORATORY EXPOSURE OF Physarum

Microplasmodia were maintained as submerged shake flask cultures in rectangular boxes; stainless steel electrodes comprise two sides of the flask (Goodman et al. 1975). Microplasmodia were exposed to continuous (24 hrs./day, 7 days/week) MSK modulated 76, 1.0 G., 1.0 V/m electromagnetic fields during the 1984 portion of the program via an IITRI-supplied function generator.

EFFECTS OF CONTINUOUS MODULATED EXPOSURE ON MITOSIS: The laboratory component of this program has been designed to provide data on the effects of various ELF-EMF exposure regimens under controlled conditions in addition to developing new protocols applicable to the field study. Control (non-exposed) cultures were placed in one incubator and the ELF-EMF cultures were maintained in another incubator. Temperature was controlled using a master-slave arrangement previously described. To perform an experiment, cultures in the log phase of growth (24 hours after transfer) were harvested, centrifuged (250 x g, 10 sec.), the old medium decanted, and the packed volume noted. The microplasmodia were quickly washed in distilled water, recentrifuged as above, decanted and resuspended in two volumes of distilled water. Two ml aliquots of this suspension were inoculated to filter paper (Schleicher and Schuell, #576, 8.2 cm) supported by stainless-steel, mesh, grids. Cultures were consecutively numbered and the code identifying the origin of the cells noted. This information was not available to the individual determining the onset of mitosis to insure that the cultures were scored in a blind manner.

The suspension was allowed to coalesce on the filter paper for 30 minutes before 17 ml of nutrient medium was added to each plate. The time of medium addition was noted (zero time); cultures were placed in the control incubator and the onset of the second or third mitosis determined.

RESULTS AND DISCUSSION-LABORATORY DATA

The first data set (Table I) shows the effect of a continuous 76 Hz, modulated 1.0G, 1.0 V/m field on the third mitosis (M-III). The microplasmodia in this data set represent day-70 of exposure through day-126. The* data show a small but statistically significant acceleration in the onset of M-III in exposed cultures $E = 24.5$ hrs. vs $C = 24.8$ hrs. (18 minutes). Although these data are statistically significant we are hesitant to claim an effect unless and until it is repeated by a duplicate experiment for several

* For an explanation of the statistical routines, see Appendices III and IV.

reasons. First, the magnitude of the difference is only 12 minutes out of approximately a 24-25 hour time period. Secondly, there is considerable scatter in each day's data set. Although our rationale for looking at M-III rather than M-II was based on the fact that inherent differences in the cell cycle would be magnified, we now find that this is also true for scatter in the data. Finally, lab experiments conducted on M-II using these same cultures (day 138-175) did not show a statistically significant difference in the length of their mitotic cycle $E=15.1$ hrs. vs $C=15.1$ hrs. (Table 2). These results are at variance with work published in 1979 (Goodman et. al. Radiat Res. 1978:485.) in which modulated field exposure delayed M-II (second mitosis), 0.4 to 0.5 hours. However, in the earlier experiments the applied fields were different 76Hz (mod), 2.0 G., 0.7 V/m, 0.4 G., 0.14 V/m, & 0.1 G. 0.035 V/m. A new set of cultures is being set up to verify these data.

EFFECT OF CONTINUOUS EXPOSURE ON QO_2 : An examination of O_2 consumption encompassing days 117 through 176 of ELF-EMF exposure (Table 3) showed no change between exposed and control cultures ($E = 0.79 \mu l O_2/\text{min}/\text{mg protein}$ vs $C = 0.79 \mu l O_2/\text{min}/\text{mg protein}$).

If these data can be reproduced they will strengthen the emerging fact that subtle changes in the applied field components can induce markedly different results. For example, the laboratory data suggest that: continuous exposure to 75 Hz. cw, 2.0 G., 0.7 V/m lengthens the mitotic cycle and decreases the respiration rate; intermittent (5 days/week, 16 hrs./day) exposure to 76 Hz. CW, 1.0 G., 1.0 V/m lengthens the mitotic cycle and increases the respiration rate, and that continuous exposure to 76 Hz (mod) 1.0G., 1.0 V/m has no detectable effect on either mitosis or respiration with the precision ($\pm 5\%$) of our methods.

FIELD EXPOSURE AT THE W.T.F.

EFFECT ON MITOSIS: Plasmodia were placed in the vicinity of the W.T.F. antenna on May 25th. Samples were routinely collected and returned to UW-Parkside for analysis of ELM-EMF effects. The mitosis data from the ground site (G) is more complex to deal with because these cultures became contaminated approximately midway through

the study and had to be restarted. The initial data (May-July, Table 4) indicates a delay in the onset of M-III in plasmodia exposed at the ground site vs controls (E = 26.1 hrs. vs C = 24.0 hrs.). Examination of the restarted cultures (July - October, Table 5) failed to show a significant difference when M-II was scored (E = 14.7 hrs. vs C = 14.8 hrs.).

The data from the A-1 site (above the buried antenna) show a delay in the onset of M-III (E = 26.7 hrs. vs C = 25.2 hrs., Table 6) and in M-II (E = 18.8 hrs. vs C = 15.7 hrs., Table 7). In both data sets the data is significant at $p < 0.01$ using both parametric and non-parametric analyses.

The data from the A-2 site (under the antenna) indicate a delay in the onset of M-III (E = 26.0 hrs. vs C = 24.3 hrs., Table 8); a significant difference was not found in the onset of M-II (Table 9). A cursory glance at the M-II data suggests that the experimental may be dividing faster than the controls. To check for the robustness of this result, we remove one day's data at a time and recompute the t-statistics; the data were not significant when subjected to this procedure.

One problem in assessing the data from the field experiments is that both M-II and M-III experiments were performed thus reducing the N in each class. The rationale for examining M-III was the assumption that differences would be magnified and easier to discern. Because of the large amount of scatter we encountered with M-III from laboratory exposure we reasoned that field data might be better if we examined M-II. In fact, this assumption was incorrect since the scatter in the field data appears to be less than we encountered with laboratory exposure. Nevertheless, field exposure to ELF-EMF in general tends to produce results that agree with those obtained from laboratory exposure, that is, exposed plasmodia show a lengthened mitotic cycle.

EFFECTS OF FIELD EXPOSURE ON RESPIRATION (OXYGEN-PROBE): Examination of the QO_2 (μ l O_2 consumed/mg protein/min) from cultures exposed at the ground site showed no statistically significant difference in their QO_2 (E = 0.55 vs C = 0.51, Table 10). Although the difference is not significant, a trend toward an elevated QO_2 in exposed cultures could be inferred. In these experiments, the low N

may in part be responsible for the apparent trend and/or the absence of a significant effect.

Examination of plasmodia exposed at the A-I site shows that ELF-EMF exposed plasmodia display a significant elevation in their QO_2 ($E = 0.65$ vs $C = 0.58$, Table 11); a similar effect was obtained at the A-II site ($E = 0.63$ vs. $c + 0.58$, Table 12). These data are significant using both parametric $p < 0.01$ using the parametric paired-t test and at $p = 0.0001$ using the non-parametric randomization test.

Collectively these field data are in agreement with the laboratory data reported last year (December 1983) showing that intermittent ELF-EMF exposure elevates the respiration rate in Physarum. The laboratory and field data have been summarized in Tables A-D.

ATP LEVELS IN MICROPLASMODIA: In view of the close metabolic correlation between O_2 consumption and ATP synthesis, we decided that it might prove fruitful to examine the level of ATP in cells exposed to weak electromagnetic fields at the W.T.F. We, therefore, investigated the possibility of assessing ATP levels in microplasmodia using a modification of techniques we currently use with haploid amoeba. Briefly, microplasmodia were placed in boiling TRIS-borate buffer (pH 9.0), the ATP extracted for 10 minutes and the suspension centrifuged at 20,000g. The supernatant was analyzed for ATP using the luciferin luciferase assay; to normalize the data, a protein determination is run on the pellet. The data obtained to date indicates that this approach is feasible and should be added to the protocols being used to determine the effects of field exposure at the W.T.F.

OXYGEN CONSUMPTION USING THE S-3A ANALYZER ON MACROPLASMODIA: To date, oxygen consumption of W.T.F.-exposed plasmodia growing on agar plates have not shown any statistically significant differences when compared to non-exposed controls. Although the data from these experiments have not been included, they do suggest a trend toward increased respiration in ELF-EMF exposed cultures. We are presently modifying our protocols to both increase the number of data in each trial and to reduce the scatter in the data.

Table A
Summary of Effects of Continuous Laboratory Exposure 76Hz (mod),
1.0G., 1.0 V/m on Mitosis

<u>Number of hours to Metaphase III</u>		Average Difference \pm SE (E-C)	<u>P</u>
Control	EMF-exposed		
24.76	24.54	-.22 \pm .16	<.01
<u>Number of hours to Metaphase II</u>		Average Difference \pm SE (E - C)	<u>P</u>
Control	EMF-exposed		
15.12	15.10	-.02 \pm .06	—

TABLE B
Summary of Effects of Continuous Laboratory Exposure (76 Hz (mod.) 1.0 G, 1.0 V/m) on
the Respiration Rate (μ l O₂ Consumed/mg. protein/min.)

<u>Respiration Rate</u>		Average Difference \pm S.E. (E - C)	<u>P</u>
Control	EMF-Exposed		
.79	.79	0 \pm .01	—

TABLE C
Summary of Effects on Mitosis From Exposing Cultures at the W.T.F.

Site	<u>Number of hours to Metaphase III</u>		Average Difference \pm S.E. (E - C)	<u>P</u>
	Control	EMF-Exposed		
G	24.00	26.06	2.06 \pm .21	<.01
A _I	25.18	26.67	1.49 \pm .22	<.01
A _{II}	24.33	26.01	1.68 \pm .21	—
Site	<u>Number of Hours to Metaphase II</u>		Average difference \pm S.E. (E-C)	<u>P</u>
	Control	EMF-Exposed		
G	14.75	14.69	-.06 \pm .16	—
A _I	15.74	18.78	3.04 \pm .66	<.01
A _{II}	16.06	15.61	-.45 \pm .17	—

TABLE D
Summary of Effects of W.T.F. Field Exposure on the Respiration Rate
(μ l O₂ consumed/mg protein/min)

Site	<u>Respiration Rate</u>		Average Difference \pm S.E. (E - C)	<u>P</u>
	Control	EMF-exposed		
G	.51	.55	.04 \pm .02	—
A _I	.58	.65	.07 \pm .01	<.01
A _{II}	.58	.63	.05 \pm .01	<.01

TABLE 1

**Summary of the Effects of Continuous Laboratory Exposure to 76 Hz, (mod.),
1.0 G., 1.0 V/m on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the third mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Experimental and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L.	AVERAGE	CONTROL	AVERAGE
4-12-84	23.25 23.25 23.05 23.5 23.52	23.31	23.35 23.67 23.48 23.18 22.78	23.29
4-16-84	24.2 23.67 24.35 24.3 23.5	24	24.28 23.87 24.75 24. 24.3	24.24
4-17-84	25.37 24.9 24.8 25.25	25.08	24.3 24.33 23.72 24.08 23.42	23.97
4-18-84	23.87 23.9 23.92 23.87 23.17	23.74	24.58 25.17 24.5 25.18	24.86
4-23-84	23.97 23.8 23.83 23.73 23.82	23.83	24.85 24.85 24.43 24.98 24.27	24.68
4-24-84	25.02 24.58 24.75 24.85 24.55	24.75	24.28 25 24.72 23.38 23.4	24.16
4-25-84	24.55 25.37 24.03 24.38 23.7	24.41	24.73 25.33 24.58 23.98 25.47	24.82
4-30-84	24.53 23.52 24.02 23.18 23.73	23.8	23.33 23.58 23.7 23.77 23.62	23.6
5-01-84	24.32 23.88 24.18 23.37	23.94	23.72 23.43 23.75 23.78 23.08	23.55

5-02-84	23.22		23.2	
	23.32		23.25	
	23.27		23.48	
	23.52	23.33	24.06	23.49
5-03-84	23.42		24.28	
	23.37		24.57	
	22.58		23.63	
	23.08		23.22	
	22.98	23.09	24.52	24.04
5-07-84	22.75		24	
	23.25		24.13	
	23.4		24.33	
	22.9		24.6	
	22.73	23.01		24.27
5-08-84	23.55		23.87	
	23.87		24.83	
	24.08		24.08	
	23.72		24.23	
	24.95	24.03	23.92	24.19
5-09-84	22.78		23.27	
	23.22		23.42	
	23.37		23.12	
	22.82		22.77	
	22.53	22.94	23.7	23.25
5-14-84	24.87		25.1	
	24.5		24.88	
	24.45		25.2	
	24.63		24.9	
	23.67	24.42	24.73	24.96
5-15-84	26.08		26.17	
	24.37		24.97	
	24.25		25.42	
	23.77		25.75	
	24.55	24.6	24.83	25.43
5-17-84	24.05		24.62	
	24.17		24.45	
	23.33		24.3	
	23.98		24.27	
	24.32	23.97	24.03	24.33
5-21-84	26.37		26.6	
	27.37		26.23	
	25.9		26.77	
	26.38		26.73	
	26.65	26.53	26.97	26.66

5-22-84	23.05		23.85	
	23.52		24.3	
	23.35		23.83	
	22.37		24.23	
	23.6	23.18	24.15	24.07
5-23-84	26.97		26.48	
	25.98		25.97	
	25.5		25.95	
	25.4		26.42	
	25.25	25.82	26.18	26.2
5-24-84	24.65		24.3	
	24.25		24.27	
	24.37		24.5	
	23.92		23.7	
	24.5	24.34	23.8	24.11
5-28-84	27.87		28	
	28.8		28.2	
	27.72		28.13	
	28.12		28.03	
	27.45	27.89	28.07	28.09
6-04-84	27.17		27.18	
	27.1		26.25	
	26.6		26	
	26.28		25.87	
	26.42	26.71	25.78	26.22
6-06-84	26.02		26.08	
	25.58		25.83	
	26.18		25.5	
	25.87		26	
	26.07	25.94	25.08	25.7
6-07-84	26.43		26.47	
	26.67		26.75	
	26.57		27.17	
	27.17	26.71	26.92	26.83
OVERALL AVERAGE	24.54		24.76	
AVERAGE DIFFERENCE		-.22		
STANDARD DEV. OF DIFF.		-.053		
NTOT =	242			
DEG. FREEDOM =	192			
T-STATISTIC =	4.2653	**		

 RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG. OF FREEDOM
1. 4-12-84	4.3142 **	184
2. 4-16-84	4.1492 **	184
3. 4-17-84	5.1915 **	185
4. 4-18-84	3.477 **	185
5. 4-23-84	3.6415 **	184
6. 4-24-84	4.8839 **	184
7. 4-25-84	4.1444 **	184
8. 4-30-84	4.4885 **	184
9. 5-1-84	4.6361 **	185
10. 5-2-84	4.2079 **	186
11. 5-3-84	3.6411 **	184
12. 5-7-84	3.3518 **	185
13. 5-8-84	4.2636 **	184
14. 5-9-84	4.0843 **	184
15. 5-14-84	3.9085 **	184
16. 5-15-84	3.9037 **	184
17. 5-17-84	4.0315 **	184
18. 5-21-84	4.2597 **	184
19. 5-22-84	3.648 **	184
20. 5-23-84	4.1127 **	184
21. 5-24-84	4.4823 **	184
22. 5-28-84	4.14 **	184
23. 6-4-84	4.7791 **	184
24. 6-6-84	4.5049 **	184

TABLE 2

**Summary of the Effects of Continuous Laboratory Exposure to 76 Hz., (mod.),
1.0 G, 1.0 V/m on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average mitotic time of the Experimental and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical condition.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPT'L	AVERAGE	CONTROL	AVERAGE
6-19-84	16.8 16.22 16.57 16.07	16.41	16.12 16.42 15.97 15.67 16.1	16.05
6-20-84	15.83 16.25 15.97 15.82	15.97	15.53 15.78 15.82 15.92 15.67	15.74
6-21-84	15.93 15.67 15.28	15.63	15.85 15.4 15.53 15.7 15.12	15.52
6-27-84	15.58 14.33 16.05 15.45 15.88	15.46	15.35 15.52 15.22 15.12 15.2	15.28
6-28-84	15.03 15.05 15.85 14.75 14.63	15.06	14.98 15.07 14.87 15.53 15.22	15.13
7-17-84	15.22 15.13 14.97	15.11	15.33 15 15 15	15.08
7-18-84	14.33 14.37 14.17 14.53 14.3	14.34	14.33 14.25 14.58 14.42 15.08	14.53
7-19-84	14.87 14.47 14.2 14.95 14.17	14.53	15.15 15.33 15.38 15.33 15	15.24
7-25-84	13.83 13.58 14 14.03	13.86	14.18 14.13 14.13 14.18 14.95	14.32

7-26-84	14.7		14	
	14.67		14.57	
	14.6		14.63	
	14.68		13.83	
	14.7	14.67	14.55	14.32

OVERALL AVERAGE:	15.10		15.12
AVERAGE DIFFERENCE:		-.02	
STANDARD DEV. OF DIFF.		.06	

NTOT =	92
DEG. FREEDOM =	72
T-STATISTIC =	.2804

*P	< .05	**P	< .01
----	-------	-----	-------

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 7-26-84	.8515	64
2. 7-25-84	.4425	65
3. 7-19-84	.8462	64
4. 7-18-84	.0185	64
5. 7-17-84	.327	67
6. 6-28-84	.1869	64
7. 6-27-84	.6482	64
8. 6-21-84	.4738	66
9. 6-20-84	.6365	65
10. 6-19-84	.878	65

TABLE 3

**Summary of Effects of Continuous Laboratory Exposure to 76 Hz., (mod),
1.0 G, 1.0 V/m on the QO_2 ($\mu l O_2$ consumed mg protein/min)
of Physarum polycephalum**

These tables compare the oxygen consumption in Control and ELF-EMFexposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Expt'l and Control cultures, the average difference and the standard deviation of the difference. This is followed by the number of data (NTOT), the Degrees of Freedom, and the T-Statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPTL.	AVERAGE	CONTROL AVERAGE	
5-29-84	.821 .805 .802	.809	.801 .832 .779	.804
6-05-84	.806 .839 .825	.809	.806 .827 .792	.809
6-08-84	.882 .846 .780	.836	.886 .746 .926	.853
6-12-84	.726 .755 .673	.718	.886 .765 .712	.764
6-13-84	.790 .714 .750	.751	.790 .758 .838	.795
6-19-84	.581 .598 .586	.589	.571 .547 .534	.55
6-20-84	.598 .590 .628	.605	.645 .640 .679	.655
6-21-84	.758 .788 .734	.760	.746 .762 .729	.746
6-22-84	.743 .807 .879	.810	.877 .865 .758	.833
6-26-84	.858 .912 .904	.891	.861 .920 .835	.872
6-27-84	.769 .818 .833	.807	.790 .756 .722	.756
6-28-84	.843 .861 .870	.858	.773 .770 .768	.77
6-29-84	.780 .769 .807	.758	.740 .741 .805	.762

7-10-84	.721		.746	
	.738		.732	
	.733	.730	.740	.739
7-11-84	.710		.673	
	.590		.692	
	.613	.637	.625	.663
7-17-84	.850		.931	
	.811		.847	
	.815	.825	.843	.873
7-19-84	.791		.987	
	.981		.919	
	.970	.914	.966	.957
7-24-84	.913		.751	
	.897		.806	
	.934	.914	.789	.782
7-25-84	.785		1.01	
	.79		.938	
	.755	.778	.971	.973
7-26-84	.928		.790	
	.928		.683	
	.933	.930	.748	.740
7-27-84	.865		.806	
	.844		.854	
	.825	.844	.826	.829
7-3-84	.793		.857	
	.758		.836	
	.762	.771	.772	.822
7-5-84	.821		.779	
	.884		.801	
	.854	.853	.781	.787
OVERALL AVERAGE:	.793		.788	
AVERAGE DIFFERENCE:		.005		
STANDARD DEV. OF DIFF.		.007		
NTOT =	138			
DEG. FREEDOM =	92			
T-STATISTIC =	.7262			

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
-----	-----	-----
1. 5-29-84	.6941	88
2. 6-05-84	.6087	88
3. 6-08-84	.9141	88
4. 6-12-84	1.0635	88
5. 6-13-84	1.0378	88
6. 6-19-84	.4776	88
7. 6-20-84	1.0548	88
8. 6-21-84	.6343	88
9. 6-22-84	.9424	88
10. 6-26-84	.6108	88
11. 6-27-84	.3985	88
12. 6-28-84	.1493	88
13. 6-29-84	.5821	88
14. 7-03-84	1.078	88
15. 7-05-84	.2958	88
16. 7-10-84	.7846	88
17. 7-11-84	.9326	88
18. 7-17-84	1.0637	88
19. 7-19-84	1.1133	88
20. 7-24-84	.146	88
21. 7-25-84	2.0294*	88
22. 7-26-84	.528	88
23. 7-27-84	.6271	88

TABLE 4
Summary of the Effects of Exposure at the W.T.F. (Ground Site)
on the Mitotic Cell Cycle of Physarum polycephalum

These tables compare the onset of the third mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and control cultures. The average difference and the *standard deviation of the difference* is followed by the number of data (NTOT), the Degrees of Freedom and the T-Statistic. A double star indicates the data are significant $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L.	AVERAGE	CONTROL	AVERAGE
6-14-84	25.88		24.45	
	25.73		22.97	
	27.17		24.23	
	26.63		24.47	
		26.35	22.82	23.79
6-25-84	25.5		24.62	
	26.47		24.92	
	27.15		25.5	
	26.75		26.67	
	26.38	26.45	26.38	25.62
7-2-84	24.72		22.77	
	25.67		22.87	
	25.55		23.38	
	25.53		23.22	
	25.48	25.39		23.06
7-9-84	26.32		23.75	
	25.55		23.98	
	26.23		23.08	
		26.03	23.37	23.55
OVERALL AVERAGE:	26.06		24.00	
AVERAGE DIFFERENCE:		2.06		
STANDARD DEV. OF DIFF.		.211		
NTOT =	35			
DEG. FREEDOM =	27			
T-STATISTIC =	9.75**			

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 6-18-84	8.5057**	20
2. 6-25-84	11.1323**	19
3. 7-2-84	7.2068**	20
4. 7-9-84	7.6374**	22

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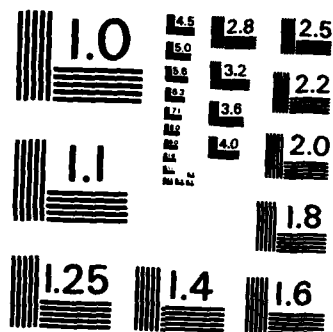
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MICROCOPY RESOLUTION TEST CHART
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TABLE 5

**Summary of the Effects of Exposure at the W.T.F. (Ground Site)
on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPT'L	AVERAGE	CONTROL	AVERAGE
8-28-84	15.08 15.08 15.08	15.08	15.5 15.42 15.92 15.08	15.48
8-29-84	15.05 14.58 14.67 15.3 14.5	14.82	14.5 14.5 15.08 16.25 15.3	15.13
8-30-84	14.5 14.08 15.08 17.2 16.42	15.46	15.78 14.42 15.37 15.45	15.25
11-5-84	14.65 14.53 14.7 14.78 14.62	14.66	14.72 15.97 14.67	15.12
11-7-84	13.53 13.87 14.68 13.95 14.07	14.02	14 13.5 13.42 14 14.17	13.82
11-8-84	14.2 14.2 14.67 13.88 13.58	14.11	13.5 13.5 13.58 13.67 14.42	13.73
OVERALL AVERAGE:	14.69		14.75	
AVERAGE DIFFERENCE:		-.06		
STANDARD DEV. OF DIFF.		.16		
NTOT =	54			
DEG. FREEDOM =	42			
T-STATISTIC =	.3989			

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 8-28-84	.0138	37
2. 8-29-84	.0856	34
3. 8-30-84	.911	35
4. 11-5-84	.0854	36
5. 11-7-84	.6201	34
6. 11-8-84	.8028	34

TABLE 6

**Summary of the Effects of Exposure at the W.T.F. (A₁ Site)
on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the third mitotic division (following addition of medium in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L	AVERAGE	CONTROL	AVERAGE
6-18-84	27.25		24	
	26.33		22.97	
	27.02		24.23	
	27.88		24.47	
		27.12	22.82	23.7
6-25-84	25.22		24.62	
	27		24.92	
	26.58		25.5	
	25.2		26.67	
	26.63	26.13	26.38	25.62
7-9-84	25.83		23.75	
	25.28		23.98	
	25.32		23.08	
	25.85		23.37	
	25.25	25.51		23.55
8-20-84	27.38		28.17	
	27.55		27.72	
	28.05		27.65	
	28.7	27.92		27.84

OVERALL AVERAGE: 26.67 25.18
 AVERAGE DIFFERENCE: 1.49
 STANDARD DEV. OF DIFF. .223

NTOT = 35
 DEG. FREEDOM = 27
 T-STATISTIC = 6.6907**

* P < .05

** P < .01

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 6-18-84	3.379**	20
2. 6-25-84	8.3439**	19
3. 7-9-84	4.6178**	20
4. 8-20-84	7.5153**	22

TABLE 7

**Summary of the Effects of Exposure at the W.T.F. (A₁ Site)
on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the second mitotic division (following addition of medium) in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L	AVERAGE	CONTROL	AVERAGE
7-30-84	21.33 23.38 22.4 22.08	22.3	22.42 12.33 23.3 12.33 23.53	18.78
8-23-84	19.37 18.58 19.8 18.35 18.67	18.95	18.08 16.67 17.32 18.43	17.63
11-5-84	15.28 15.85 16.55 15.6	15.82	14 14 13.75 13.75 13.75	13.85
11-6-84	19.45 18.43 16.73 18.45 17.08	18.03	14.22 15.47 14.17	14.62
11-7-84	18.98 18.35 19	18.78	14 13.5 13.42 14 14.17	13.82
OVERALL AVERAGE: 18.78				
AVERAGE DIFFERENCE: 3.04				
STANDARD DEV. OF DIFF. .661				
NTOT = 43				
DEG. FREEDOM = 33				
T-STATISTIC = 4.5985**				
* P < .05				
** P < .01				

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 7-30-84	12.7721**	26
2. 8-23-84	4.1862**	26
3. 11-5-84	3.9605**	26
4. 11-6-84	3.7146**	27
5. 11-7-84	3.1667**	27

TABLE 8

**Summary of the Effects of Exposure at the W.T.F. (A-II Site)
on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the third mitotic division (following addition of medium in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L	AVERAGE	CONTROL	AVERAGE
6-18-84	27.43 28.3 27.82 27.83	27.85	24.45 22.97 24.23 24.47 22.82	23.79
6-25-84	25.5 25.63 26.25 27	26.1	24.62 24.92 25.5 26.67 26.38	25.62
7-2-84	24.18 24.28 23.95 24.28 24.78	24.3	24.1 24.62 24.45	24.39
7-9-84	26.38 25.65 25.55 26.43 25.03	25.81	23.75 23.98 23.08 23.37	23.55
OVERALL AVERAGE:	26.01		24.33	
AVERAGE DIFFERENCE:		1.68		
STANDARD DEV. OF DIFF.		.208		
NTOT =	35			
DEG. FREEDOM =	27			
T-STATISTIC =	8.0528**			

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 6-18-84	3.7757**	20
2. 6-25-84	10.0155**	20
3. 7-2-84	8.6639**	21
4. 7-9-84	5.8526**	20

TABLE 9

**Summary of the Effects of Exposure at the W.T.F. (A-II Site)
on the Mitotic Cell Cycle of Physarum polycephalum**

These tables compare the onset of the second mitotic division (following addition of medium in Control and EMF exposed (Expt'l) plasmodia. The summaries show the overall average time of Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPT'L.	AVERAGE	CONTROL	AVERAGE
8-23-84	16.92		18.08	
	16.13		16.67	
	15.3		17.32	
	15.92		18.43	
	15.8	16.01		17.63
8-28-84	15.45		15.5	
	15.72		15.42	
	16.38		15.92	
	15.73		15.08	
	16.18	15.89		15.48
8-29-84	14.5		14.5	
	15.13		14.5	
	14.5		15.08	
	14.5		16.25	
	15.13	14.75	15.3	15.13
8-30-84	15.03		15.78	
	17.05		15.37	
	16.22		15.45	
	16.85	16.29		15.53
10-8-84	15.53		16.05	
	14.92		16.83	
	15.03		16.37	
	14.92		16.88	
		15.1	16.45	16.52
OVERALL AVERAGE:	15.61		16.06	
AVERAGE DIFFERENCE:		-.45		
STANDARD DEV. OF DIFF.		.17		

NTOT = 44
 DEG. FREEDOM = 34
 T-STATISTIC = 2.7062**

* P < .05 ** P < .01

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 8-23-84	.9109	27
2. 8-28-84	3.3455**	27
3. 8-29-84	2.5029*	26
4. 8-30-84	4.4483**	29
5. 10-8-84	1.0213	27

P = .01 for T-test by randomization

TABLE 10

**Summary of the Effects of Exposure at the W.T.F. (Ground Site)
on the QO_2 ($\mu l O_2$ consumed mg protein/min)
of Physarum polycephalum**

These tables compare the oxygen consumption in Control and ELF-EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXP'T'L	AVERAGE	CONTROL	AVERAGE
7-3-84	.556 .447 .595	.534	.562 .79 .746	.699
7-10-84	.659 .623 .596	.626	.633 .606 .586	.608
8-30-84	.418 .536	.477	.428 .393	.410
8-29-84	.548 .601 .546	.565	.428 .393	.410
8-31-84	.506 .538 .529	.524	.389 .468 .370	.409

OVERALL AVERAGE: .545 .507
 AVERAGE DIFFERENCE: .04
 STANDARD DEV. OF DIFF. .02

NTOT = 27
 DEG. FREEDOM = 17
 T-STATISTIC = 1.6447

* P < .05 ** P < .01

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 7-3-84	5.2809**	13
2. 7-10-84	1.4754	13
3. 8-30-84	1.2332	15
4. 8-29-84	.313	14
5. 8-31-84	.6472	13

TABLE II
Summary of the Effects of Exposure at the W.T.F. (A-I Site)
on the QO_2 ($\mu l O_2$ consumed mg protein/min)
of Physarum polycephalum

These tables compare the oxygen consumption in Control and ELF-EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPT'L	AVERAGE	CONTROL	AVERAGE
6-5-84	.861 .826	.844	.378 .441	.410
7-10-84	.482 .510	.496	.633 .606 .586	.608
8-3-84	.642 .635 .556	.611	.406 .415 .451	.424
8-22-84	.738 .727 .716	.727	.622 .58 .526	.576
8-23-84	.635 .634 .639	.636	.402 .447 .414	.421
8-24-84	.517 .572 .583	.558	.464 .453 .466	.461
10-9-84	.575 .616 .645	.620	.734 .723 .723	.726
10-10-84	.584 .620 .527	.577	.759 .740 .773	.758
10-11-84	.688 .730 .806	.741	.759 .817 .836	.804

OVERALL AVERAGE: .645 .583
 AVERAGE DIFFERENCE: .062
 STANDARD DEV. OF DIFF. .009

NTOT = 51
 DEG. FREEDOM = 33
 T-STATISTIC = 7.3349**

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 6-5-84	2.3423*	31
2. 7-10-84	9.0292**	30
3. 8-3-84	5.5103**	29
4. 8-22-84	5.8806**	29
5. 8-23-84	4.7954**	29
6. 8-24-84	6.359**	29
7. 10-9-84	8.9307**	29
8. 10-10-84	10.1161**	29
9. 10-11-84	9.4817**	29

P = .0009 for T-test by randomization

TABLE 12

Summary of the Effects of Exposure at the W.T.F. (A-II Site)

on the QO_2 ($\mu l O_2$ consumed mg protein/min)

of Physarum polycephalum

These tables compare the oxygen consumption in Control and ELF-EMF-exposed (Expt'l) microplasmodia. The summaries show the overall average QO_2 for Experimental and Control cultures. The average difference and the standard deviation of difference is followed by the number of data (NTOT), the Degrees of Freedom, and the T-statistic. A double star indicates the data are significant at $p < .01$, the level we require before accepting an EMF effect.

The second data set recomputes the T-statistic after removing one day's data at a time. The purpose of this routine is to insure that a single day difference does not bias the final statistical conclusion.

Finally, the data are subjected to the non-parametric randomization test where the p value is recomputed following 10,000 data permutations.

DAY	EXPT'L	AVERAGE	CONTROL	AVERAGE
7-3-84	.712 .708 .603	.674	.562 .790 .746	.699
7-10-84	.590 .545 .513	.549	.633 .601 .586	.608
7-20-84	.564 .626 .597	.596	.664 .732 .816	.737
8-3-84	.630 .663 .640	.645	.406 .415 .451	.424
8-23-84	.604 .662	.633	.402 .447 .414	.421
8-24-84	.571 .595 .604	.590	.464 .453 .466	.461
8-28-84	.539 .483 .535	.519	.501 .514 .557	.524
8-29-84	.565 .551 .681	.599	.428 .393	.410
8-30-84	.669 .745 .677	.697	.389 .468 .370	.409
8-31-84	.536 .501 .378	.472	.488 .470 .491	.483
10-9-84	.671 .685 .629	.662	.734 .723 .723	.726
10-10-84	.730 .627 .696	.685	.759 .740 .773	.758
10-11-84	.743 .862 .822	.809	.759 .817 .836	.804

10-12-84	.677		.60	
	.768		.672	
	.728	.724	.573	.615

OVERALL AVERAGE:	.632		.581
AVERAGE DIFFERENCE:		.051	
STANDARD DEV. OF DIFF.		.01	

NTOT =	82
DEG. FREEDOM =	54
T-STATISTIC =	5.1944**

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME

REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. 7-8-84	6.3661**	50
2. 7-10-84	5.6727**	50
3. 7-20-84	6.4886**	50
4. 8-3-84	3.7296**	50
5. 8-23-84	3.8666**	51
6. 8-24-84	4.3321**	50
7. 8-28-84	5.2921**	50
8. 8-29-84	4.1733**	51
9. 8-30-84	3.376**	50
10. 8-31-84	5.5764**	50
11. 10-8-84	5.6546**	50
12. 10-10-84	5.8103**	50
13. 10-11-84	5.3843**	50
14. 10-12-84	4.6283**	50

P = .0001 for T-test by randomization.

APPENDIX I

Location and electromagnetic field intensity measurements at the sites used to determine the effects of exposure to the W.T.F. antenna.



View of the west antenna ground right of way, looking south to west ground pole #N15 (stockade).



View of study plot with the buried culture chamber in place.

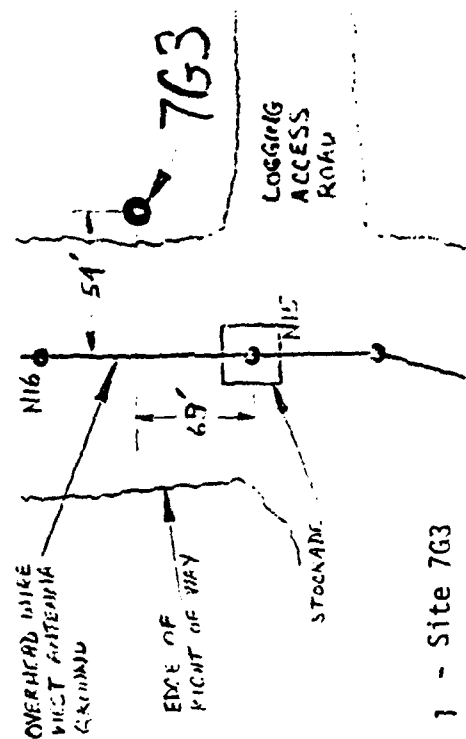
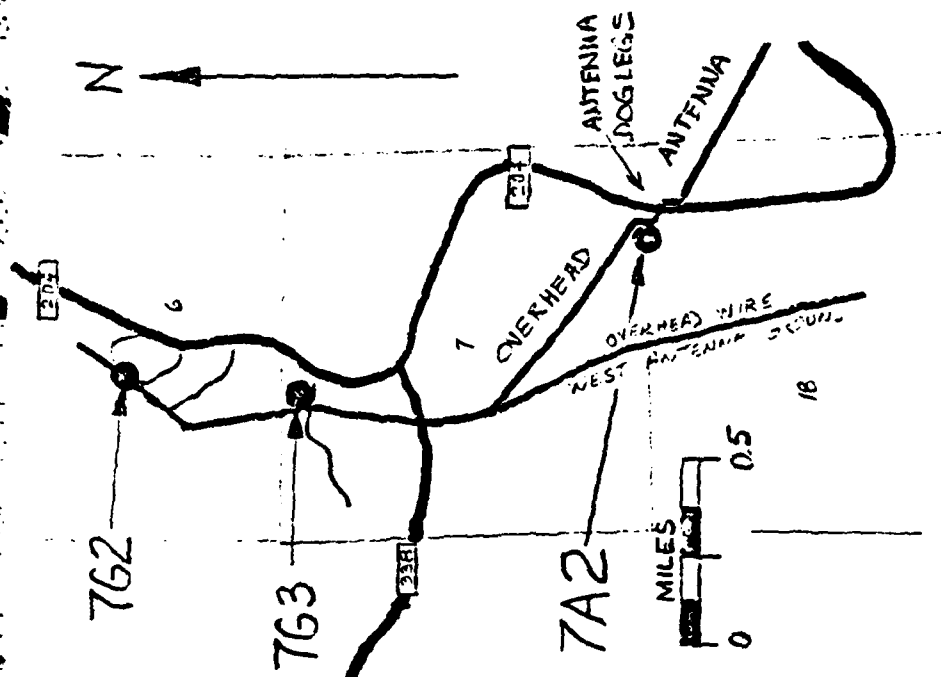


Figure 1. Site 763. 1 - Site 763

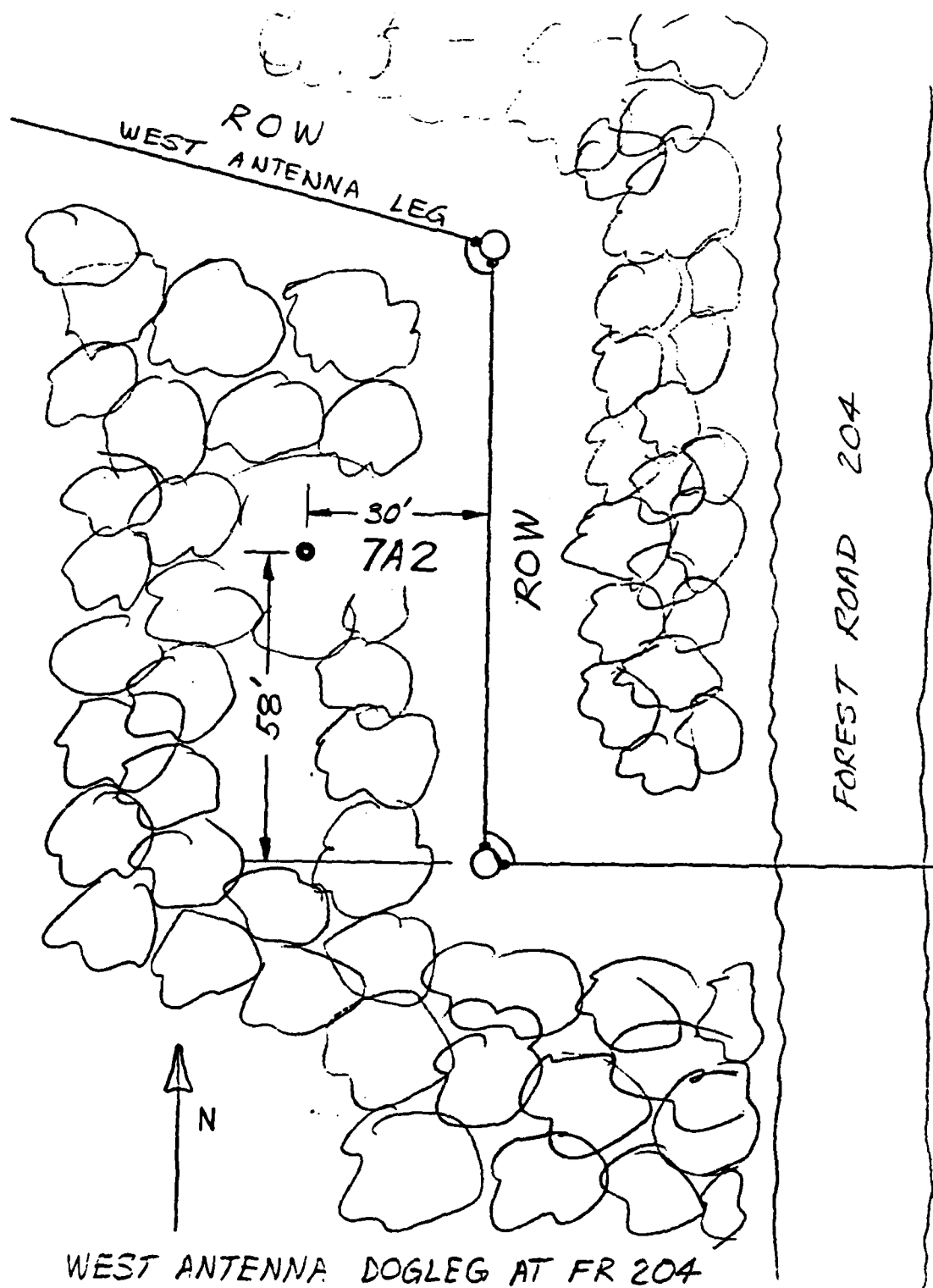


Figure 2.

Table 1

External Electric Field Intensities and
Magnetic Flux Densities¹

Site No.	Meas. Pt.	Meas. Yr.	Transverse E Field (Air) (V/M)		Longitudinal E Field (Earth) (mV/M)		Magnetic Flux Density (mG)	
			76 Hz	60 Hz	76 Hz	60 Hz	76 Hz	60 Hz
7A1	1	82	--	--	188	0.092	184	0.033
7A1	1	83 ^A	--	--	157	0.116	163	0.077
7A1	1	83 ^B	--	--	164	0.13	149	0.019
7A1	1	84	0.17	<0.001	156	0.11	153	0.030
7A2	1	84	--	--	204	0.035	45	0.002
7A2	1	84	0.035	<0.001	239	0.052	314	<0.001
7C1	1	82	--	--	1.8	0.062	0.026	0.002
7C1	1	83	--	--	1.9	0.070	0.025	<0.001
7C1	1	84	--	--	2.2	0.099	0.025	<0.001
7G3	1	83	--	--	1860	0.091	5.2	<0.001
7G3	1	84	1.5	<0.001	1510	0.13	5.6	0.001

1 Values shown are magnitudes determined as the square root of the sum of the squares of the orthogonal field components measured.

A Before hole was dug.

B After hole was dug.

Table 2

Ratio Magnitudes For External Fields

Compared: Site No's.	Transverse Electric Field (Air)				Longitudinal Electric Field (Earth)				Magnetic Field			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
A1/C1	170	170	170	1.000	71	1418	1576	1.111	6120	5100	153000	30.000**
A2/C1	>35	>35	>35	1.000	>93	>3923	>2061	0.354-0.525	>1800	>22500	>45000	1.000-2.000
G3/C1	1500	15000	15000	1.000	686	11615	15253	1.313	224	5600	5600	1.000

- 1) R1 = Test Site (76 Hz) / Control Site (76 Hz) R1 > = 10.
 R2 = Test Site (76 Hz) / Test Site (60 Hz) R2 > = 10.
 R3 = Test Site (76 Hz) / Control Site (60 Hz) R3 > = 10.
 R4 = Test Site (60 Hz) / Control Site (60 Hz) 0.1 < = R4 < = 10.

When a range of values were available for calculating a given ratio, the ratio range was calculated as follows:

High Limit ---> Maximum Numerator Value / Minimum Denominator Value
 Low Limit ---> Minimum Numerator Value / Maximum Denominator Value

** Does not meet the exposure criterion.

Table 3
Culture Chamber and Earth Electric
Field Intensities (76 Hz)

Site No.	Chamber	Meas. Yr.	Chamber Voltage (mV)	Chamber E Field (mV/m)	Longitudinal E Field (Earth) (mV/m)
7A1	West(A)	84	4.8	30.97	166.25±14.93
	East(B)		13.5	87.10	
7A2	West	84	3.0	19.35	221.50±24.75
	East		4.2	27.10	
7C1	#1	84	0.030	0.19	1.97±0.21
	#2		0.010	0.06	
7G3	North	84	14.5	93.55	1,685±247.49
	South		16.7	107.74	

1 Mean ± standard deviation for 1982 - 1984 data, see Table 1.

Table 4

Exposure Ratio For Culture Chamber

Compared:
Chamber No's.

Electric Fields

	R1	R2	R3	R4
T1/C1-1	160	4800	4364	0.909
T1/C2-2	480	4800	9600	2.000
T2/C1-1	450	13500	12273	0.909
T2/C1-2	1350	13500	27000	2.000
T3/C1-1	100	3000	2727	0.909
T3/C1-2	300	3000	6000	2.000
T4/C1-1	140	4200	3818	0.909
T4/C1-2	420	4200	8400	2.000
T5/C1-1	483	14500	13182	0.909
T5/C1-2	1450	14500	29000	2.000
T6/C1-1	557	16700	15182	0.909
T6/C1-2	1670	16700	33400	2.000

- 1) R1 = Test Site (76 Hz) / Control Site (76 Hz)
 R2 = Test Site (76 Hz) / Test Site (60 Hz)
 R3 = Test Site (76 Hz) / Control Site (60 Hz)
 R4 = Test Site (60 Hz) / Control Site (60 Hz)

R1 > = 10.
 R2 > = 10.
 R3 > = 10.
 0.1 < = R4 < = 10.

- * T1 = 7A1 West(A)
 T2 = 7A1 East(B)
 T3 = 7A2 West
 T4 = 7A2 East
 T5 = 7G3 North
 T6 = 7G3 South
 C1-1 = 7C1
 C1-2 = 7C1

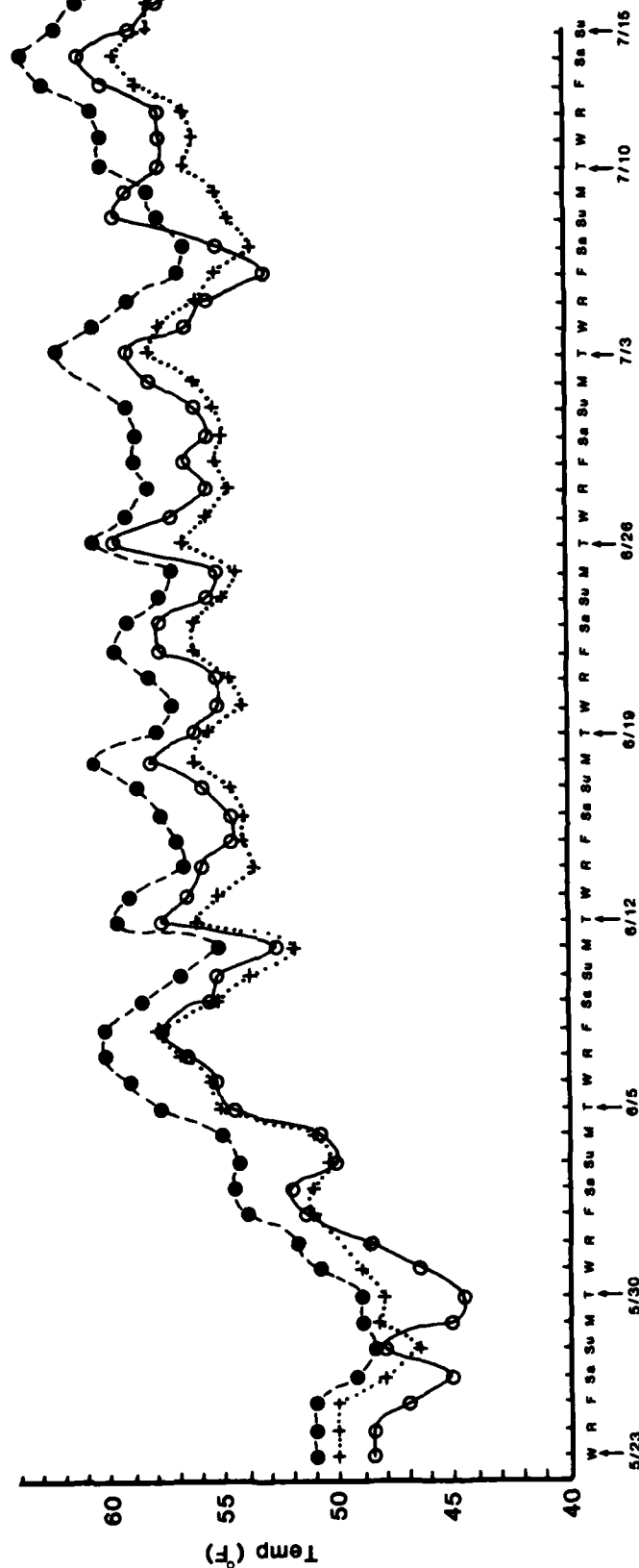
Note: |E| in chamber for test site at 60 Hz was assumed to be 0.001 mV/m.

APPENDIX II

Daily Record of Temperature at Field Sites

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Temperature Graphs 5/23 - 7/15

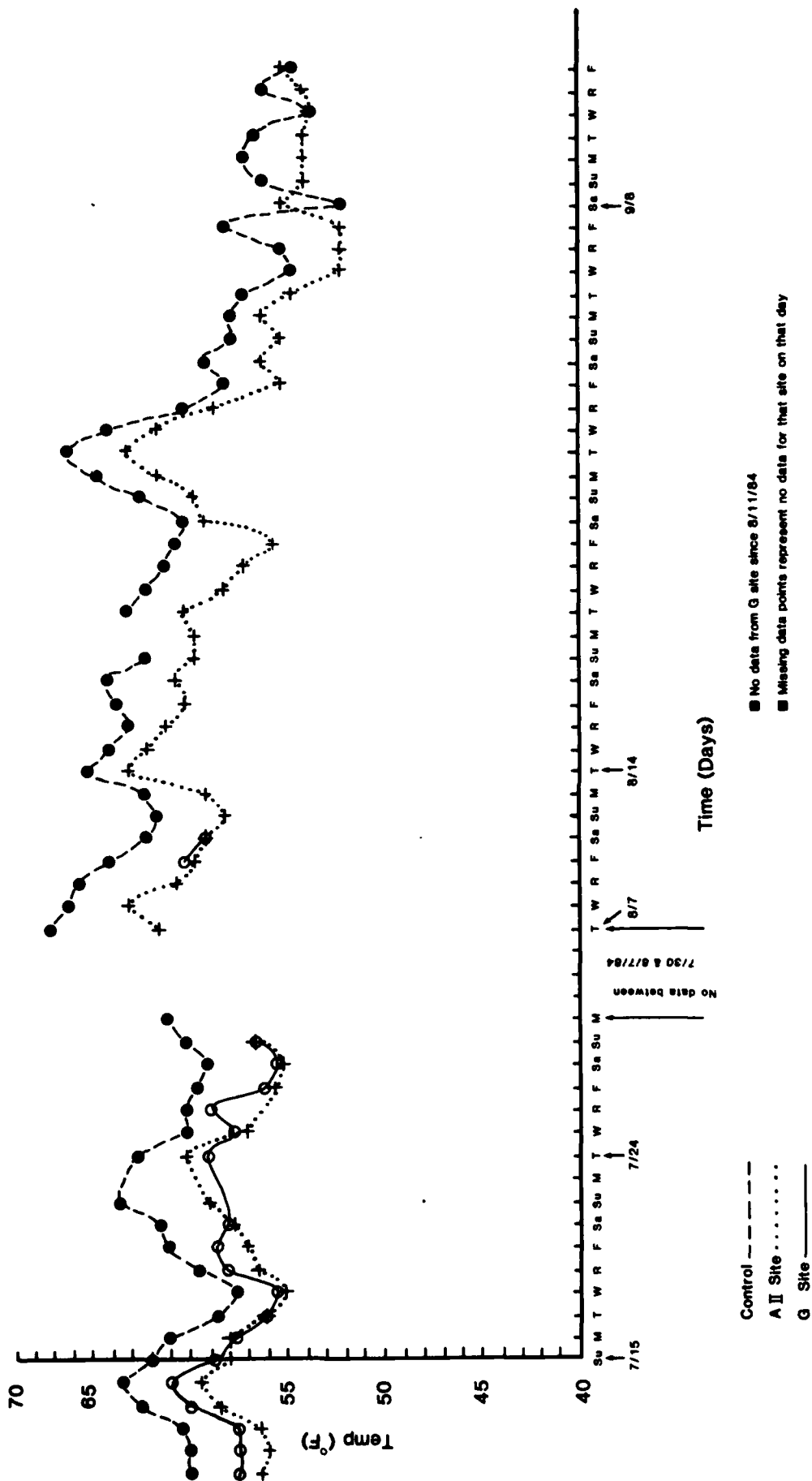


Time (Days)

Control ---
 All Site
 G Site —

■ Missing data points represent no data for that site on that day

Temperature Graphs 7/15 - 9/14



APPENDIX III

Statistical Analysis of Laboratory and Field Data

Analysis of Data: We employ two techniques for statistical analysis, a parametric paired t-test, and a non-parametric randomization test. The parametric test is fast and used only as an indicator of when a full non-parametric test is warranted. The non-parametric computation on the APPLE computer requires approximately one hour for a preliminary computation of 1000 trials, and overnight for a full 10,000 trials.

The parametric paired t-test is described below. Note that if the data is all collected on a single day, the variance computed in item 2 is the "conventional" paired-t variance. Item three outlines how the variance is estimated for more than one day's data. The crucial point of course is the number of degrees of freedom assigned to the statistic when assessing its significance. This is given in item 4. Note here that we employ a conservative estimate of degrees of freedom from the point of view of being able to describe a difference as being statistically significant. This can be seen by noting that if only one control value and one experimental value are measured on each day, we would assign zero degrees of freedom to the statistic.

It must be emphasized that the parametric computation is not the one we rely on for significance of an effect. It is used only as a quick and approximate measure of the statistical significance of a difference. The randomization test is relied upon to assess the significance of an observed difference, and this test does not require that we assign degrees of freedom to the computed statistic. We have enclosed a listing of the program we used to perform this computation together with a sample listing using hypothetical data (Appendix IV). In this example, the parametric measure of significance is $p = 0.024$ and the non-parametric measure is $p = 0.034$. We regard this agreement as excellent, especially in view of the small number of data used in this example.

Example: STATISTICAL ANALYSIS
OF MITOSIS DATA USING
HYPOTHETICAL DATA

4/84

Parametric Computation

MITOSIS DATA ANALYSIS FOR DATA TYPE = M2

DAY/ [DIFF.]	EXP'T'L.	AVERAGE	CONTROL	AVERAGE
DAY ONE ** (ZERO=6)	15 (9)		14 (8)	
	15.5 (9.3)		14.5 (8.3)	
	16 (10)		15 (9)	
	15 (9)		15 (9)	
[.6]	15.5 (9.3)	[15.4]	15.5 (9.3)	[14.8]
DAY TWO ** (ZERO=6.3)	15 (9.3)		13 (7.3)	
	14.5 (9)		14.5 (9)	
	14.25 (8.45)		13.5 (8)	
	15.5 (10)		14.5 (9)	
[.7625]		[14.81]	14.75 (9.15)	[14.05]

Start @ 6:30pm
1st Control @ 7:30am
1st Exptl @ 9:30am

OVERALL AVERAGE: 15.1063 14.425
AVERAGE DIFFERENCE: .6812 hr
STANDARD DEV. OF DIFF. .2718

NTOT= 19
DEG. FREEDOM= 15
T-STATISTIC= 2.5068*

* P<.05 ** P<.01

← 4.7% diff E > C

Actual value of P = 0.024

RECOMPUTE T-STATISTIC REMOVING ONE DAY AT A TIME		
REMOVE DAY	T-STATISTIC	DEG OF FREEDOM
1. DAY ONE	1.6754	7
2. DAY TWO	1.8974	8

check for Robustness of parametric computation. This would not be considered a robust result. Mkt, however, the n is small.

COMPUTE P-VALUE FOR T-TEST BY RANDOMIZATION

1000 PERMUTATIONS OUT OF 31752 POSSIBLE
NG=3 AFTER 153 PERMUTATIONS AND P=.0196078431
NG=11 AFTER 294 PERMUTATIONS AND P=.037414966
NG=13 AFTER 412 PERMUTATIONS AND P=.0315533981
NG=20 AFTER 569 PERMUTATIONS AND P=.0351493849
NG=23 AFTER 705 PERMUTATIONS AND P=.0326241135
NG=33 AFTER 942 PERMUTATIONS AND P=.0350318471
FOUND 34 PERMUTATIONS WITH GREATER T-STATISTICS

INTERMEDIATE Readouts
requested by keyboard
query during computation

P=.034 FOR T-TEST BY RANDOMIZATION

Note reasonable agreement with
parametric computation

P=.034 vs P=.024

METHOD FOR COMPUTATION OF PAIRED T-TEST

1. The best estimate of the mean square variability for a given day, i , is obtained by pooling variation in control and experimental observations. Thus,

$$MS_i = \frac{SS_{ci} + SS_{Ei}}{(\eta_{ci} - 1) + (\eta_{Ei} - 1)} \equiv \frac{SS_i}{N_i - 2}$$

Note: $SS_i \equiv SS_{ci} + SS_{Ei}$

$$N_i \equiv \eta_{ci} + \eta_{Ei}$$

2. The variability for Δ_i on a given day is obtained by adding estimated variance for control and experimental cultures from that day:

$$\sigma = \frac{MS_i}{\eta_{ci}} + \frac{MS_i}{\eta_{Ei}} = MS_i \left(\frac{\eta_i}{\eta_{ci} \cdot \eta_{Ei}} \right) = \frac{N_i SS_i}{(N_i - 2)(\eta_{ci} \eta_{Ei})}$$

By definition, then

$$MS_i \equiv N_i \sigma_i^2 = \frac{N_i^2 SS_i}{(N_i^2)(\eta_{ci} \cdot \eta_{Ei})}$$

3. The estimate for the variance of the overall difference, $\bar{\Delta}$, is obtained by computing a weighted average of the daily mean square variations,

$$MS_{TOT} = \frac{\sum (N_i - 2) MS_i}{\sum (N_i - 2)}$$

$$\sigma_{TOT}^2 = \frac{MS_{TOT}}{\sum N_i} = \frac{\sum \frac{N_i^2 SS_i}{(\eta_{ci} \cdot \eta_{Ei})}}{\sum N_i \sum (N_i - 2)}$$

4. The t-statistic with $(\sum N_i - 2 \times \text{number of days})$ degrees of freedom is

$$t = \bar{\Delta} / \sigma_{TOT}$$

Symbol Definitions:

SS_{ci} = sum of squares for control data for day i

SS_{Ei} = sum of squares for experimental data for day i

n_{ci} = number of control data on day i

n_{Ei} = number of experimental data on day i

N_i = total number of data for day i

Δ = average difference between control and experimental for day i

$\bar{\Delta}$ = overall average difference for all days

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1. Cover Page:

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c. Title: ELF communications System Ecological Monitoring
Program, Task 5.2, Soil Amoeba.

d. Reporting year: November 1, 1983 to October 31, 1984.

2. Frontispage:

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d. Reporting year: November 1, 1983 to October 31, 1984.

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3. Table of Contents:

1. Cover Page.....	i
2. Frontispage.....	iii
3. Table of Contents.....	v
4. Abstract.....	vii
5. Summary.....	ix
6. Progress Report.....	1
a. Introduction.....	1
b. Work Plan.....	3
c. Experimental.....	9
7. Peer Reviewers.....	17
8. Tables and Figures.....	18

4. Abstract:

Experimental and control study sites, identified in the 1983 field season, were used. In 1983, the sites were characterized as to electromagnetic background, physical and chemical properties, and biological characteristics. Some of this work was continued into the 1984 season.

Studies of soil amoebae in 1983 were designed to provide sufficient data to determine sample sizes and methods of statistical analysis suitable for comparing control and experimental sites. These were utilized in the 1984 field season. Control and experimental sites contained the same species of amoebae, total number of amoebae was the same by statistical analysis at the sites and growth rates in soil submersible culture vessels was statistically the same at the sites for a species of soil amoeba commonly isolated from the sites.

5. Summary:

Amoebae are common soil organisms present in large numbers (i.e. ca. 4,000/g soil). Ecologically their role in soil formation from organic matter (i.e. mineralization) is to eat bacteria and fungi. They are also capable of preying on multicellular organisms including man, although this is not common. In turn, amoebae are eaten by others, notably fungi and bacteria. A gram of soil typically contains at least three species of amoebae (which add up to 4,000 individuals) so that they are doing different things together in a small soil unit.

As cells, they are used extensively in Cell Biology in place of or in conjunction with cells from animals including man. Examples can be seen in cancer research, studies of muscle contraction, developmental biology and molecular biology. This is possible because their basic structure and function is the same as liver cells, heart cells, or any other cell from higher animals. Consequently any physiological effects induced by ELF on cells of higher organism would also affect soil amoebae.

Any ecological stress induced by electromagnetic effects of ELF would be reflected in detectable changes in the soil amoeba population. Changes would include numbers of active amoebae, species diversity, their physiology and their distribution in soil. Consequently, the activity of amoebae in soil away from the antenna will be compared with amoebae from similar soils near the antenna and ground wire.

In the 1983 field season, control and experimental sites were identified which resembled each other biologically, physically and chemically. In addition the final sites were monitored for ambient electric and magnetic effects in soil by IITRI. Criteria for electromagnetic exposure were established by IITRI. Background electromagnetic radiation was found acceptable for control, antenna and ground wire sites. Sampling and growth experiments were done to determine variability and statistical methods appropriate for comparing control and experimental sites.

In the 1984 field season the full work plan was implemented. The primary thrust was to demonstrate that the control, antenna and ground sites were biologically similar. The soil submersible cultures, used to determine growth activity at the sites, were performed without electrical input since the antenna was not installed. Species and strain characterization was expanded to include an isoenzyme analysis of Acanthamoeba polyphaga clones from the study sites. Population size and activity was determined throughout the season; a peak was noted in August when the population exceeded 1 million/g soil.

6. Progress report:

INTRODUCTION

Soil amoebae play a significant role in soil mineralization, acting as micro-predators and serving as food for other soil organisms. Since several soil amoebae are used in cell and molecular biology, much of the biochemical and physiological mechanisms underlying their biology is known. Thus, possible electromagnetic (EMF) effects on animal cells would be readily detected in these organisms.

Possible stress induced by EMF might act directly on the organism. Goodman, E.M., Greenbaum, B., Marron, M.T. (1976. Rad. Res. 66, 431-40) published evidence of an effect on the length of the interphase portion of the cell cycle and cytoplasmic streaming of the slime mold Physarum, an amoeboid organism. Friend, Jr., A.W., Finch, E.D., Schwan, H.P. (1975. Science 187, 357-59) found that the freshwater amoeba Chaos chaos oriented to an EMF. Thus, EMF's may exert effects on basic cell functions. An EMF could also exhibit indirect effects by acting on other soil components important to the amoebae. The National Research Council (1977. Report of the Committee on Biosphere Effects of Extremely-Low-Frequency Radiation) recommended that studies on amoebae be followed up and that a general ecological study be done on the effects of EMF of extremely low frequency (ELF). The present research is part of that effort.

2.

Environmental changes in soil that take place over time (e.g. moisture, temperature, etc.) are important to this study. Electromagnetic effects might be restricted to a biological state induced by a particular environment (e.g. vegetative vs. dormant amoebae). Obviously environmental changes will directly effect soil amoebae as well. Experimental sites (antenna and ground wire) are compared to a control site, located 9 miles south of the antenna, and all sites are studied over the growing season. In addition, data accumulated for each growing season, before the antenna is installed will form a base line to compare to sites after the antenna is operational.

OBJECTIVES: For the 1984 field the primary objective was to demonstrate that the control, antenna and ground wire study sites were biologically similar in regards to soil amoebae. In addition a base line was accumulated for comparison with future data, especially that obtained once the antenna is operational.

WORK PLAN ELEMENTS: the following was presented in the Technical Proposal for 84/85, dated January 11, 1984:

#0. Plot selection and characterization.

Synopsis: this is element #2 of the FY82/83 work plan. The study plot at Ground Wire site #4 has not been selected. A tentative site has been chosen but it has not been surveyed by IITRI for background electromagnetic radiation to see if it is comparable to the control and antenna sites. This was not done in 1983 because Ground Wire site #4 was not identified until late in the season.

#1. Species and strain characterization.

Synopsis: using morphological and physiological markers, identify species and strains of soil amoebae from the study areas so that possible changes in the population due to ELF can be detected.

Specifics: see Technical Discussion for over-all rationale and restatement of FY 82/83 objectives and work elements. Species of soil amoebae present at the study sites are isolated from soil enrichment plates. Comparisons can then be made of species composition between sites. For the species Naegleria gruberi, a common soil-dwelling organism, physiological changes within populations will be examined by isozyme analysis and by restriction fragment analysis of mitochondrial DNA. Present

observations have failed to reveal species differences so far between sites or between soil horizons at each site. A statistical analysis of species diversity is not practical. The soil dilution technique yields data that is too scattered to recognize a dominant species. The number of plate wells positive for a given species are too few, for most of the season, to provide adequate data on individual species number. Counts of single species may be possible in 1985 if the population again reaches large numbers.

Without a genetic analysis, the isoenzyme patterns have to be considered as having a mixture of different genetic origins. These are not allozymes as defined by Prakash, S., Lewontin, R.C. and Hubby, J.L. (1969. Genetics 61, 841-58). Consequently data analysis cannot utilize techniques used in population genetics to present and analyze the data. How the data is to be analyzed will have to wait until I determine the frequency of electrophoretic variants in the isolates. Initial analysis will utilize Rf's of each isoenzyme from a given isolate.

This work plan element is being performed at MSU.

#2. Population size and activity.

Synopsis: determine population size of amoebae in soil and the ratio of vegetative to dormant amoebae over the growing season. This is a productivity measure which could be affected by ELF, it could also be a reflection of changes in the microbial food organisms due to ELF.

5.

Specifics: an established soil dilution counting technique is used, in which a soil sample is diluted 15 fold and eight replicate cultures are made from each dilution. In order to count vegetative amoebae and cysts, samples are first divided in half, one half is used to count total cysts and vegetative amoebae while the other half is treated to kill vegetative amoebae so that only cysts are counted. Differential counts can be used to calculate by subtraction the total vegetative amoeba count. In the 1982 season I found that 10 random samples, subdivided into organic and mineral horizons, provided statistically adequate data. Of course this meant 10 samples X 2 horizon, X 15 dilutions, X 8 replicates, X 3 sites --a significant effort. Two-way analysis of variance between sites and dates for each horizon was a satisfactory statistical approach. The soil horizons at each site differed between themselves, as anticipated

#3. Growth and feeding activity.

Synopsis. determine the in situ growth and feeding activity of amoebae in soil submersible culture vessels. This will provide data on growth rate, feeding activity and mean generation time (i.e. the cell cycle time between nuclear mitoses).

Rationale: the technique involves suspending a known amoeba species, previously isolated from the study sites, in a physiological saline with a food bacterium. Direct counts of amoebae are made with a microscope to determine increase in number of organisms and nuclei over time. A log transform of

6.

this data provides a straight line plot which can be determined with a regression analysis. Statistically significant differences between slopes can be detected with 95% Confidence Limits of the line, a version of the t-test. The method of counting amoebae is more accurate than soil dilution techniques so that fewer replicates are needed. Last season I found that 4 replicates are adequate. This approach will be used to determine growth rate and thus mean generation time. The mean generation time will be comparable to the cell cycle measurement of time between mitoses of Physarum. Cropping activity will be determined by varying the number of bacteria available for amoeba growth and then following growth rate and maximum yield of amoebae over time.

#4. Ambient monitoring.

Synopsis: automatic monitoring of soil temperature and moisture was performed during part of the 1983 field season. Both measures were useful for general trends. Soil moisture techniques used for automatic, remote monitoring suffer from an equilibration lag after a rain. It is important to make a direct measurement of soil moisture at the time soil samples are taken for differential counts between vegetative amoebae and cysts. Amoebae require a soil moisture content of 0.3 bar suction for vegetative growth.

#5. Data analysis.

Synopsis: statistical analyses mentioned earlier in the work plan include procedures necessary to test for differences between sites and between sampling dates. Implied but not stated was the need to separately compare soil horizons since these differ in the number of organisms but not in species composition. The moisture binding properties of soil horizons differ so that they can differ in supporting vegetative growth of microorganisms on that basis alone. A two-way analysis of variance, using 10 samples per horizon at each site was found to be adequate in comparing sites for population density and %vegetative vs. cysts.

Growth measurements in soil submersible culture vessels were best analyzed by comparing slopes of regression lines calculated from log transformed cell counts taken over time. The 95% confidence limit of the slope is actually a form of t-test and is a satisfactory method for comparing slopes between growth curves. This will be used to compare vegetative activity at sites, to compare mean generation times (= duration of the interphase time between mitoses in the cell cycle) and cropping activity (i.e. by varying density of food bacterium).

Ambient monitoring (temperature/moisture) will be done at each site, with probes in the organic and mineral horizons plus the interface between these. The data accumulated will be stored on micro-computer discs and hard copies. Data is condensed from 1 hr. averaging (of 5 min. reading intervals) to 4 hr. and once a day averages on the micro-computer.

The mainframe computer will be used for statistical analysis but not for the storage of ambient monitoring data--it is too expensive.

[illegible]

SCHEDULE OF WORK ELEMENTS (Nov 1 to Oct 31 each year)

[illegible]

EXPERIMENTAL

Methods and results will be presented in reference to the Work Plan, given above.

0. Plot selection and characterization. The study plot at Ground Wire Site #4 was surveyed by IITRI early in the season so that a ground wire site was available which satisfied the background electromagnetic radiation criteria (i.e. comparable to the control and antenna sites).

Since there was no significant difference in total amoeba numbers between sites, soil characteristics were not as critical as they might be if biological differences had been noted. A change in soil characteristics in future years could affect soil-dwelling micro-organisms however. Table 1 shows the chemical properties of the organic and mineral horizons for the control, antenna and ground wire sites, with replicates. Individual data points are given in Table 1 since there is not enough data to warrant the use of descriptive statistics. Table 2 demonstrates the difference in bulk density between the organic and mineral horizons; this is important when comparing amoeba numbers between horizons, and, precludes such a direct comparison. Table 3 demonstrates the slightly acidic nature of the soil in a northern hardwood forest, without significant differences between horizons or sampling dates. Comparing the 1983 and 1984 seasons indicates a slight increase in acidity. A long-term comparison will be needed before this can be considered a valid trend vs. worker differences.

1. Species and strain characterization. Species of soil

amoebae present at the study sites were isolated from soil enrichment plates. So far no species differences have been noted between sites; species composition was the same as in the 1983 field season. Species included Acanthamoeba castellanii, A. polyphaga, A. astronyxis (small strain), Hartmannella sp., Rosculus sp., Naegleria gruberi, Vahlkampfia sp., and Mayorella sp. A new amoeba-flagellate, referred to earlier, has not been submitted for publication yet. I discussed my observations on this organism last summer with several colleagues and the consensus was that it should be described as a new genus and species, however the flagellate stage needs better documentation. So far I have been unable to induce mass flagellate transformation in sufficient quantity to do electron microscopy. Before the 1985 field season, I plan to publish the description with or without electron microscope pictures of the flagellate stage (I do have EM pictures of the amoeboid and cyst stages). I will submit it as Kalavalia balamuthi, but for this report it will be called Vahlkampfia sp. since it loosely fits into this genus.

For the isoenzyme analysis, I have chosen A. polyphaga rather than N. gruberi. A. polyphaga is no more common in soil isolates than other amoebae but its cyst is very distinctive which makes it easy to pick out from soil dilution, enrichment plates (see #2 below). After making several clone isolates of this species from the study sites, I am now in the process of doing the isoenzyme analyses. The results will not be available until the March 1985 meeting. I am using the same isoenzymes as those used by the American Type Culture Collection (Daggett, P. &

Nerad, T.A. 1983. Procedures for isoenzyme electrophoretic analysis, American Type Culture Collection, 2nd. ed.). As a control, I am also using 2 strains of this species from the ATCC in the isoenzyme study. Included in the isoenzymes are some that have been used by others, e.g. DeJonckheere, J.F. 1982. Isoenzyme patterns of pathogenic and non-pathogenic Naegleria sp. using agarose isoelectric focusing. Ann. Microbiol. (Inst. Pasteur) 133, 319-42. In addition I will use Superoxide Dismutase, an enzyme used at the CDC-Atlanta for small amoebae (personal communication)svara) and some that are popular in other fields (e.g. glutamate-oxaloacetate transaminase, glyceraldehyde-3-phosphate dehydrogenase, alpha-glycerophosphate dehydrogenase and fructokinase). Anticipating the complete results, I do find some differences between clone isolates for the enzymes analyzed so far. This brings up the need to demonstrate that the isolates all fall within the definition of the species. If I can demonstrate that the cyst structure is the same for the clones and that there is overlap between isoenzyme patterns, this should provide sufficient evidence that I am dealing with strains of the same species. Since these are asexual organisms, I cannot do a genetic analysis to know which isoenzyme variants are allelic.

2. Population size and activity. From the 1983 field data, given in last year's annual report, the number of replicate samples used to determine population size at each study site (i.e. 10) was more than adequate since the coefficient of variation was <10% of the mean. For 3 sites, 10 samples, 1 date and 9 D.F., a significant difference at the 90% probability level

would be $1.4 \times \text{S.D.}$ (from a power curve); for 8 samples per site this would drop a little to 1.5 to $1.6 \times \text{S.D.}$ For the 1984 field season I chose to use 8 replicates per site since there was little loss in power between 10 and 8 replicate samples per site. Thus Darbyshire's 96 multiwell adaptation of Sing's soil dilution method (Darbyshire, J.F., Wheatley, R.E., Greaves, M.P. and R.H.E. Inkson 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. *Rev. Ecol. Sol.*, 11, 465-75) works well and has the added advantage of being useful in easily detecting and isolating species from the soil isolates. It should be kept in mind however that the Fisher & Yates Table VIII.2. DENSITIES OF ORGANISMS ESTIMATED BY THE DILUTION METHOD, used by both Singh and by Darbyshire et al. was not generated statistically but rather derived from an arithmetic equation. Therefore, both historically and in mathematical origin this is not "just an MPN method" as stated by one of last year's reviewers. In the same paper Darbyshire et al. does use an MPN method to estimate bacterial numbers. A very nice paper that should be read carefully. In the 1983 field season I killed vegetative amoebae for differential counts with sodium dodecyl sulfate, a technique used earlier in my laboratory (e.g. Umeche, N. 1983. The numbers of Naegleria spp. in Michigan soils and litters. *Arch. Protistenk.* 127, 127-30). Because it is essential that all of the detergent be removed from the soil before cultivation, I decided it would be safer to use the earlier 10% HCl technique of Sing's (Cutler's, Darbyshire's, etc.) in which 10% is actually 10% of conc. HCl. The acid can be easily removed from the soil and easily checked with a pH meter.

The results of the 1984 season indicate that the control, antenna and ground wire sites have the same number of amoebae/g soil in both the organic and mineral horizons (Table 4, Table 4B, Figs. 1 to 5). Data on the distribution of amoebae between the cyst and vegetative stages indicates that a large proportion of amoebae are in the dormant state for much of the season and vegetative amoeba distribution does differ between sites (Table 4A, Table 4B, Figs. 1, 2, 3, 6 & 7). Specifically, Table 4 gives total counts of vegetative amoebae and cysts while Table 4A gives counts of cysts alone, thus the mathematical difference gives the number of vegetative amoebae present in a sample. Figs. 1, 2 and 3 represent Tables 4 and 4A in showing total counts and cyst counts by horizon and site at various sampling dates. Fig. 4 and 5 compare total counts by horizon, which do not differ significantly (Table 4 and Table 4B). The mathematically calculated number of vegetative amoebae, given in Figs. 6 & 7, deserve attention aside from significant differences between sites. Note the counts on 8/6, which exceed a million per gram soil. This was not a sudden event since a gradual increase in numbers was detected in samples taken on 7/9 and 7/18. I will go back to this in section #4 below.

The collapse in the total amoeba population, when vegetative amoebae predominate, indicates that the vegetative form is being actively destroyed in soil. This is consistent with the population collapse noted by Clarholm (1981) (cited below). Direct studies of cyst degradation have been published (e.g. Barrett, R.A. and Alexander, M. 1977. Resistance of cysts of amoebae to microbial decomposition. *Appl. Env. Microbiol.* 33,

670-74. Verma, A.K. et al. (1974. J. Gen. Microbiol. 80, 307-9) isolated and fungus capable of destroying amoeba cysts in conjunction with a bacterial isolate. No evidence exists concerning predators of vegetative amoebae.

3. Growth and feeding activity. Without using the electrical input that will eventually be available, when the antenna is operational, I did do growth rate experiments in the soil submersible culture vessels that will be used when the antenna is operational. Growth experiments were done at the 3 sites, with 3 replicate cultures each so that growth counts were done in the field. The data given in Table 5 (and plotted in Fig. 8), a form of t-test, indicates that the 95% confidence limits overlap between sites so that there is no difference between the growth rate slopes at the 3 sites, with a mean generation time of 8 hr.

I hope to do some growth experiments in 1985 "under the wire" at the Clam Lake antenna in Wisconsin. Thanks to IITRI personnel, the electrical components of the growth vessels will be ready, and the same personnel originally set up their circuit design. A saline solution that electrically resembles the low conductivity of soil would be too dilute to support growth of amoebae (and many other micro-organisms) although soil water by itself is a satisfactory saline. The saline I use (LS-saline) is similar to soil water. The following conductivities were measured by IITRI personnel:

- a. organic horizon, soil: 0.0033 mhos/m
- b. mineral horizon, soil: 0.0016 mhos/m.
- c. LS-saline: 0.44 mhos/m.

The higher resistance of soil vs. LS-saline reflects the liquid phase discontinuities of soil. In order to mimic the electromagnetic effects in soil with LS-saline, two separate culture vessels will be needed, one to mimic voltage and the other for current. Thus at a given site, 6 culture vessels will be needed, 3 for voltage and 3 for current.

4. Ambient monitoring. Table 6 contains two different experiments. The first part of Table 6 gives the mean % (w/w) moisture for individual measurements, taken when the soil was sampled, for each set of 8 replicate samples per horizon/site/date. The second half of Table 6 gives the % moisture at 0.3 bar suction for the organic and mineral horizons at the 3 sites. By calculation, amoebae need soil capillaries filled with soil water at the level of 0.3 bar suction (I justified this last year based on the work of Darbyshire, J.F. 1975. In Walker, N. (ed.) Soil Microbiology. Halstead Press). Thus 54% water in the organic horizon and 19% water in the mineral horizon would be optimum for amoeba growth. This is based on an equilibrium situation without extremes of drying or wetting. The very wet soils of 7/18 do not correspond to the significant increase in vegetative amoebae first noted on 7/9 and peaking on 8/6. It will be interesting to see the 1985 field season pattern of soil moisture vs. growth. Although fluctuating moisture content has been demonstrated to be important to amoebae in soil (e.g. Bryant, R.J., Woods, L.E., Coleman, D.C., Fairbanks, B.C., McClellan, J.F. and Cole, C.V. 1982. Interactions of bacterial and amoebal populations in soil microcosms with fluctuating moisture content. Appl. Env. Microbiol. 43, 747-52;

Clarholm, M. 1981. Protozoan grazing of bacteria in soil--impact and importance. *Microb. Ecol.* 7, 343-50), the moisture fluctuations at the study site may not be great enough to exhibit a comparable effect.

Automated data on soil moisture (Fig. 9, 10, 11) indicate that the organic horizon is more variable than the underlying mineral horizon at a single site (Fig. 9) and between organic horizons at the three sites (Fig. 10) as opposed to the mineral horizons (Fig. 11). In the 1985 field season, I plan to place all of the data loggers at the same horizon for each site (i.e. the interface between the organic and mineral layers) in order to get replicate measurements at each site. Possibly this might yield more useful information.

Temperature exhibits minor variations throughout a 24 hr. period (Fig. 12) thus the rest of the data is plotted as a daily average (Fig. 13, 14, 15). Aside from the small temperature spread between sites, horizons, and between horizons at a site, the most interesting biological observation is the contrast between soil temperatures where microorganisms are growing and laboratory temperatures used to grow them. Commonly 30 deg. C is used in the laboratory and 20 deg. C would be frigid. To avoid genetic selection of isolates, I have lowered my laboratory incubators to approximate the summer highs of 18 to 19 deg. C.

7. Peer reviewers:

I plan to use the following individuals as peer reviewers:

- a. Prof. Thomas J. Byers
Department of Microbiology
Ohio State University
- b. Prof. Fredrick L. Schuster
Department of Biology
Brooklyn College

TABLE 1. SOIL CHEMISTRY:*

SITE/HORIZON**

Element	CO	AO	GO	CM	AM	GM
P	39,29 34,--	38,33 24,--	38,29 32,--	62,71 64,75	60,52 44,60	25,20 20,35
K	122,84 126,--	122,122 122,---	110,80 141,--	42,51 36,36	32,28 17,23	21,21 24,24
Ca	1645,1600 2779,----	2134,1778 2569,----	2000,2653 2624,--	520,520 590,590	520,520 253,295	680,379 694,379
Mg	122,178 119,---	147,157 131,---	135,197 192,---	63,62 52,40	40,17 12,16	25,57 69,16
Zn	38,44 70,--	46,42 80,--	56,38 45,--	2,3 3,3	2,2 4,2	2,2 3,3
Fe	15,15 8,--	10,15 8,--	10,15 12,--	55,50 50,63	35,40 35,33	35,30 26,23
Mn	151,149 227,---	207,252 321,---	210,252 310,---	7,8 8,7	3,5 18,14	5,5 19,5
Cu	5,5 5,-	6,5 5,-	9,10 6,--	1,1 1,1	1,1 1,1	1,1 1,1
Na	26,28 14,--	17,18 22,--	23,33 22,--	11,7 47,23	8,8 47,17	19,18 38,9
Cl	0,8 0,-	0,0 0,-	0,0 0,-	8,8 0,0	0,0 16,8	16,16 0,0
nitrate	10,19 14,--	17,26 7,--	23,36 18,--	4,0 0,0	6,0 3,2	0,0 0,0
%Org.N	22,17 14,--	14,15 13,--	13,15 13,--	2,1 1,1	1,1 1,1	1,1 2,1

* Performed by Michigan State University Soil Testing Laboratory, data expressed as ppm except for %org.N.

** SITE: C, control; A, antenna; G, ground.
HORIZON: O, organic; M, mineral.
Each datum was obtained by pooling separate horizons taken from 20 random samples per site.

TABLE 2. BULK DENSITY (g dry wgt./cc):*

SITE	HORIZON	MEAN \pm SD
Control	Organic	0.39 \pm 0.09
	Mineral	1.44 \pm 0.06
Antenna	Organic	0.44 \pm 0.1
	Mineral	1.42 \pm 0.07
Ground	Organic	0.32 \pm 0.07
	Mineral	1.36 \pm 0.09

One-way ANOVA, ORGANIC HORIZONS:

	D.F.	M.S.
Between	2	0.02754
Within	21	0.0082
F = 3.371 (N.S.)		

One-way ANOVA, MINERAL HORIZONS:

	D.F.	M.S.
Between	2	0.0148
Within	21	0.00637
F = 2.304 (N.S.)		

Mean ratio of bulk densities:

$$\text{Mineral/Organic} = 1.4098/0.3858 = 3.65$$

*From 8 replicate samples taken from each site/horizon.

TABLE 3. SOIL pH:

SITE	HORIZON	MEAN pH \pm SD (n=10) (sampled on 2 dates)
Control	Organic	6.26 \pm 0.35
	Mineral	6.11 \pm 0.34
Antenna	Organic	6.0 \pm 0.32
	Mineral	6.04 \pm 0.16
Ground	Organic	6.09 \pm 0.18
	Mineral	6.18 \pm 0.12

One-way ANOVA:

	D.F.	M.S.
Between	5	0.0894
Within	54	0.0689
F = 1.298 (N.S.)		

One-way ANOVA for 2 sampling dates (03SEP84 & 01OCT84)

	D.F.	M.S.
Between	1	0.0125
Within	28	0.0946
F = 0.132 (N.S.)		

Table 4. Total counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil [*] ± S.E.	MEAN (#/g soil)
Control	Organic	6/18	9.7539 ± 0.1738	17,221
		6/27	7.8792 ± 0.3414	2,642
		7/9	11.3533 ± 0.3795	85,246
		7/18	11.4197 ± 0.4539	91,099
		8/6	14.2648 ± 0.4357	1,567,198
		9/3	8.3852 ± 0.2014	4,382
		10/2	8.7314 ± 0.1621	6,194
	Mineral	6/18	8.1088 ± 0.2734	3,324
		6/27	7.5047 ± 0.3015	1,817
		7/9	10.5626 ± 0.2191	38,662
		7/18	11.1415 ± 0.1873	68,975
		8/6	13.9181 ± 0.3984	1,108,036
		9/3	7.4492 ± 0.0651	1,718
		10/2	7.1308 ± 0.0983	1,250
Antenna	Organic	6/18	9.6252 ± 0.3729	15,142
		6/27	7.4282 ± 0.0873	1,683
		7/9	11.0022 ± 0.2755	60,006
		7/18	11.6615 ± 0.3787	116,018
		8/6	14.6355 ± 0.2179	2,270,476
		9/3	8.6155 ± 0.2243	5,517
		10/2	8.1509 ± 0.3263	3,466
	Mineral	6/18	7.7793 ± 0.2102	2,391
		6/27	7.7391 ± 0.2663	2,296
		7/9	10.2525 ± 0.3249	28,353
		7/18	10.7765 ± 0.2440	47,882
		8/6	14.1536 ± 0.1006	1,402,266
		9/3	6.5874 ± 0.9640	726
		10/2	7.3913 ± 0.1579	1,622
Ground	Organic	6/18	9.2519 ± 0.2158	10,424
		6/27	8.0434 ± 0.1269	3,113
		7/9	11.3700 ± 0.3073	86,682
		7/18	10.8289 ± 0.2339	50,458
		8/6	14.6718 ± 0.1259	2,354,408
		9/3	8.1456 ± 0.2168	3,448
		10/2	8.0830 ± 0.2053	3,239
	Mineral	6/18	8.3214 ± 0.3488	4,111
		6/27	7.2803 ± 0.2150	1,451
		7/9	10.9209 ± 0.1509	55,321
		7/18	10.8409 ± 0.2126	51,067
		8/6	14.0038 ± 0.0575	1,207,183
		9/3	7.5718 ± 0.1639	1,943
		10/2	7.0941 ± 0.0717	1,205

* Mean expressed as the natural log of amoeba number, used to calculate analysis of variance (Table 4B).

Table 4A. Cyst counts from 8 samples per horizon/site:

SITE	HORIZON	DATE	MEAN #/g soil [*] ± S.E.	MEAN (#/g soil)
Control	Organic	6/18	6.9786 ± 0.1381	1,073
		6/27	7.8688 ± 0.1721	2,614
		7/9	7.7053 ± 0.2283	2,220
		7/18	12.1002 ± 0.1438	179,908
		8/6	11.6502 ± 0.4054	114,714
		9/3	7.3023 ± 0.1388	1,483
		10/2	7.9563 ± 0.0719	2,853
	Mineral	6/18	8.1877 ± 0.3427	3,596
		6/27	7.7400 ± 0.2712	2,298
		7/9	7.0612 ± 0.0686	1,166
		7/18	11.7853 ± 0.2285	131,308
		8/6	10.66350 ± 0.2215	42,766
		9/3	6.2443 ± 0.0739	515
		10/2	7.1632 ± 0.1373	1,291
Antenna	Organic	6/18	7.8498 ± 0.2736	2,565
		6/27	8.1367 ± 0.2135	3,418
		7/9	7.2226 ± 0.1342	1,370
		7/18	10.2111 ± 0.3522	27,203
		8/6	11.5344 ± 0.4090	102,171
		9/3	6.7868 ± 0.1448	886
		10/2	8.0375 ± 0.2207	3,095
	Mineral	6/18	7.5381 ± 0.2898	1,878
		6/27	7.5222 ± 0.1380	1,849
		7/9	6.8349 ± 0.0829	929
		7/18	10.3047 ± 0.2459	29,873
		8/6	10.8859 ± 0.1450	53,402
		9/3	6.2846 ± 0.0756	536
		10/2	7.5485 ± 0.1686	1,898
Ground	Organic	6/18	7.5293 ± 0.1922	1,862
		6/27	7.6287 ± 0.2642	2,056
		7/9	8.2238 ± 0.1488	3,729
		7/18	9.0026 ± 0.5237	8,124
		8/6	11.9692 ± 0.1882	157,818
		9/3	6.7038 ± 0.1207	815
		10/2	7.9514 ± 0.1749	2,840
	Mineral	6/18	7.4515 ± 0.3566	1,722
		6/27	7.0453 ± 0.1012	1,147
		7/9	6.7277 ± 0.0989	835
		7/18	9.4737 ± 0.2189	13,013
		8/6	11.2533 ± 0.1142	77,134
		9/3	6.2765 ± 0.1380	532
		10/2	6.9521 ± 0.0887	1,045

* Mean expressed as the natural log of amoeba number, used to calculate analysis of variance (Table 4B).

Table 4B. One-way analysis of variance by date and horizon, data transformed to ln (see Table 4 & 4A).

TOTAL COUNT					
HORIZON	DATE	GROUPS	DF	MS	F*
ORGANIC	6/18	among	2	0.544056892	
		within	21	0.575709525	0.945019787 NS
	6/27	among	2	0.811731339	
		within	21	0.374032952	2.17021344 NS
	7/9	among	2	0.345079422	
		within	21	0.838251023	0.411665972 NS
	7/18	among	2	1.46747065	
		within	21	1.07801869	1.36126643 NS
	8/6	among	2	0.405770302	
		within	21	0.675187792	0.600973991 NS
	9/3	among	2	0.441511869	
		within	21	0.367677825	1.2008118 NS
10/2	among	2	1.01611972		
	within	21	0.4663808	2.17873402 NS	
MINERAL	6/18	among	2	0.596842766	
		within	21	0.879854702	0.678342418 NS
	6/27	among	2	0.811731339	
		within	21	0.374032952	2.17021344 NS
	7/9	among	2	0.895180702	
		within	21	0.470327196	1.90331478 NS
	7/18	among	2	0.30366993	
		within	21	0.37286159	0.814430711 NS
	8/6	among	2	0.113640785	
		within	21	0.458944775	0.247613202 NS
	9/3	among	2	2.30243111	
		within	21	2.56135657	0.89891081 NS
10/2	among	2	0.208487034		
	within	21	0.106056985	1.965802 NS	
CYST COUNT					
ORGANIC	6/18	among	2	1.3810997	
		within	21	0.283298944	4.87506124 **
	6/27	among	2	0.517599344	
		within	21	0.386567729	1.3389616 NS
	7/9	among	2	2.00581956	
		within	21	0.246055944	8.1518842 **
	7/18	among	2	19.4982219	
		within	21	1.11750703	17.4479636 **
	8/6	among	2	0.405668736	
		within	21	0.978949547	0.414391872 NS
	9/3	among	2	0.841283798	
		within	21	0.146166188	5.75566626 *
10/2	among	2	0.0187032223		
	within	21	0.22531598	0.083008858 NS	
MINERAL	6/18	among	2	1.29532909	
		within	21	0.876257306	1.47825198 NS
	6/27	among	2	1.01003504	
		within	21	0.274244059	3.68297874 *
	7/9	among	2	0.231899738	
		within	21	0.0569419861	4.07256147 *
	7/18	among	2	10.9687614	
		within	21	0.428371157	25.6057423**
	8/6	among	2	0.709644794	
		within	21	0.221824601	3.19912576 NS
	9/3	among	2	0.003653764	
		within	21	0.0814418793	0.04486346 NS
10/2	among	2	0.731487513		
	within	21	0.147003537	4.9759858 *	

* = 5% significance level
 ** = 1% significance level

TABLE 5. Regression calculations for growth of Vahlkampfia sp., log transformed, in soil submerged culture vessels .

Site**	Slope*** (MGT)	95% Confidence Limits for slope	Correl. Coef.
C	0.0362 (8)	L = 0.022654 L = 0.049686	0.9855
A	0.0374 (8)	L = 0.026027 L = 0.048678	0.9851
G	0.0363 (8)	L = 0.015640 L = 0.056998	0.9716

*Data from 3 cultures/site

**SITE: C, control; A, antenna; G, ground.

***MGT = mean generation time (hr).

TABLE 6. SOIL MOISTURE (% w/w)*:

HORIZON:	CONTROL SITE		ANTENNA SITE		GROUND SITE	
	ORG	MIN	ORG	MIN	ORG	MIN
DATE:						
6/18	52±6	16±2	46±7	9±3	50±10	17±5
6/27	53±7	16±2	46±11	12±3	52±9	15±2
7/9	37±16	16±2	36±12	8±2	34±4	11±2
7/18	78±8	42±3	79±13	35±2	79±7	32±3
8/6	49±8	11±4	44±10	7±4	37±13	12±1
9/3	48±12	17±1	33±10	12±3	35±6	12±2
10/2	48±5	18±3	32±5	13±1	43±9	16±3

* Sample size: organic, 8; mineral, 8.

ORGANIC HORIZON, SOIL SUCTION, 0.3 BAR = 54%
ONE-WAY ANOVA:

	D.F.	M.S.
Between	2	62.7142868
Within	18	40.2698381

F = 1.55735135 NS

MINERAL HORIZON, SOIL SUCTION, 0.3 BAR = 19%

	D.F.	M.S.
Between	2	4.66666412
Within	15	3.54444428

F = 1.31661377 NS

FIGURE 1. Control site total counts and cyst counts.

CONTROL SITE (1984)

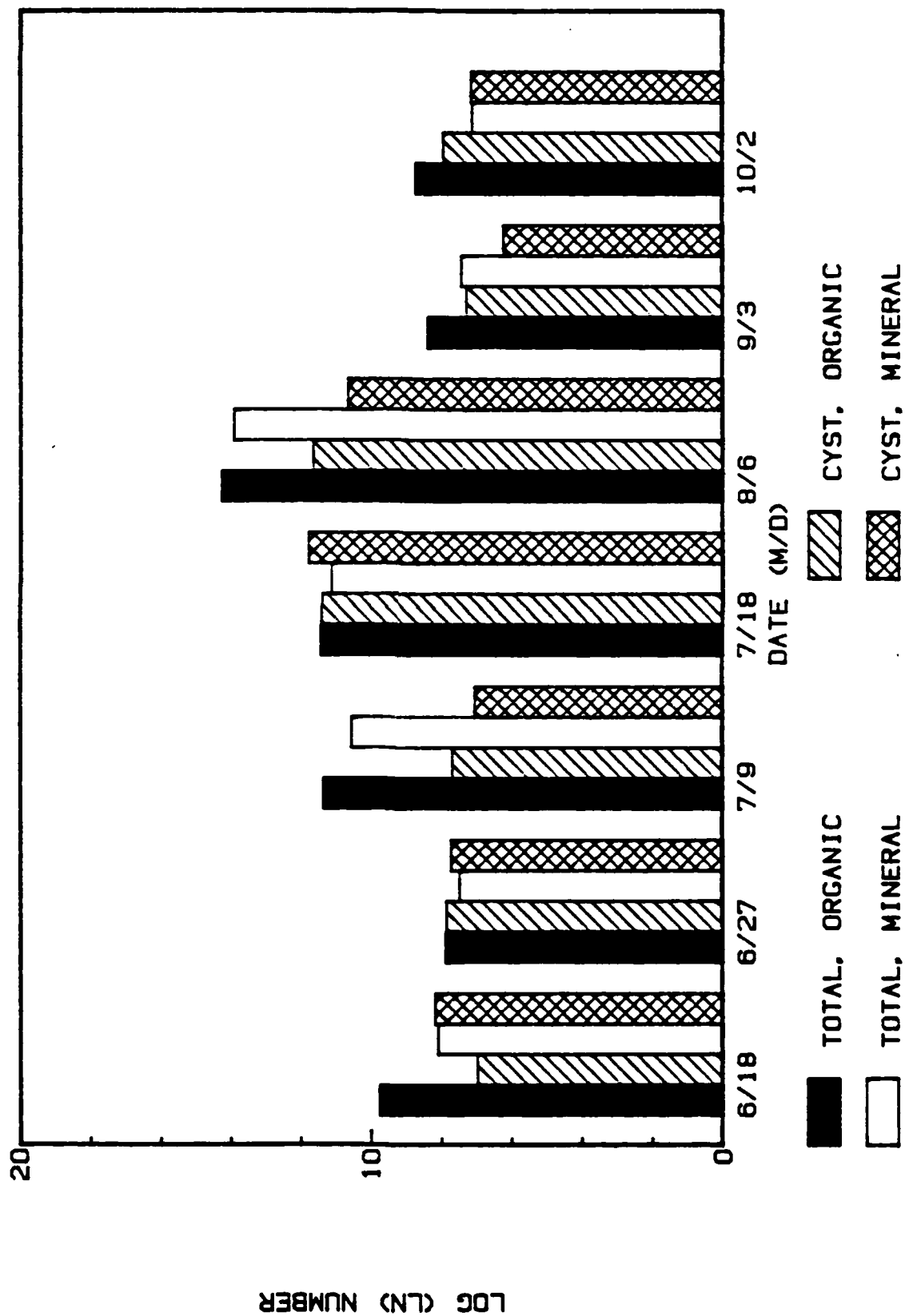


FIGURE 2. Antenna site total counts and cyst counts.

ANTENNA SITE (1984)

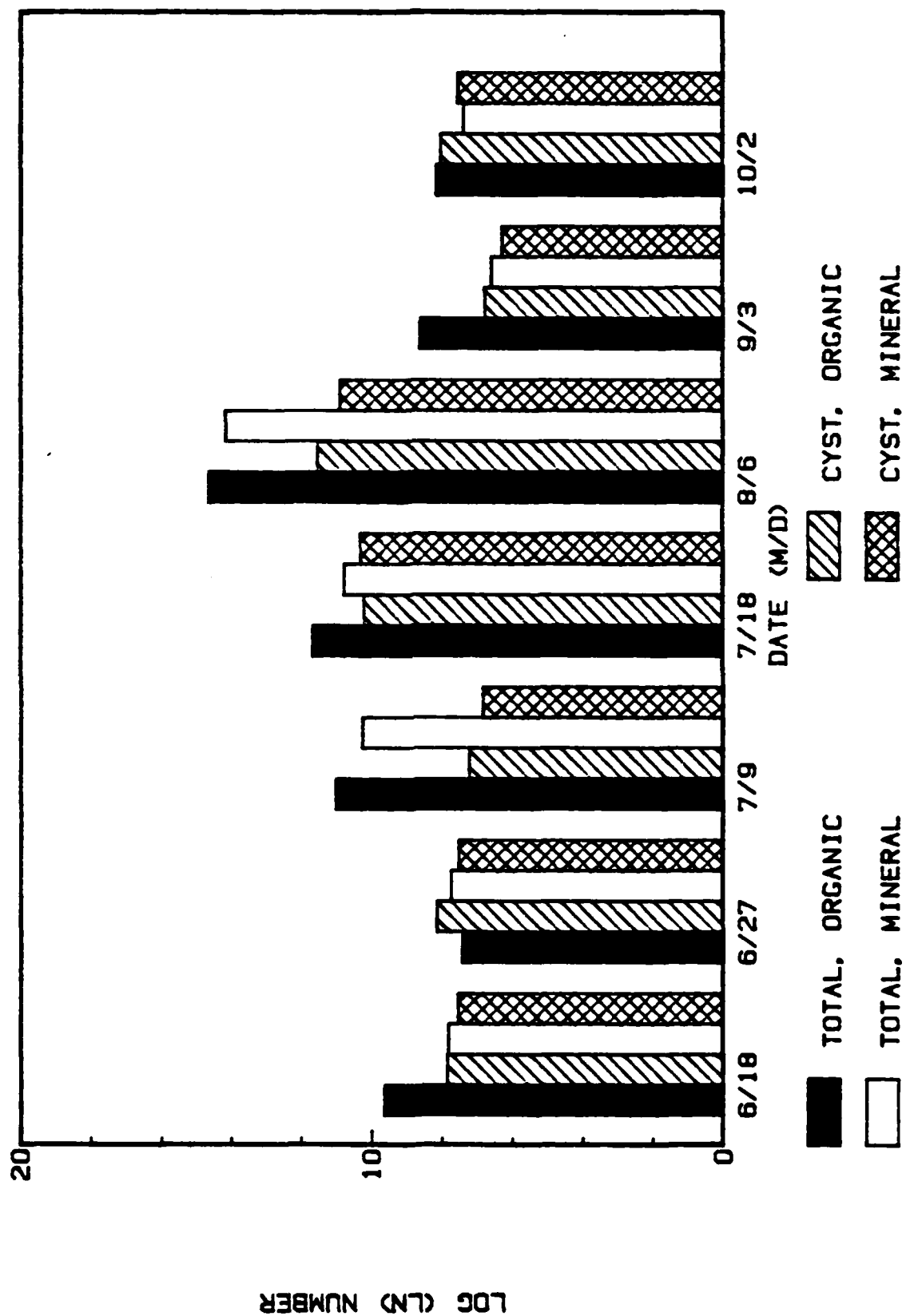


FIGURE 3. Ground site total counts and cyst counts.

GROUND SITE (1984)

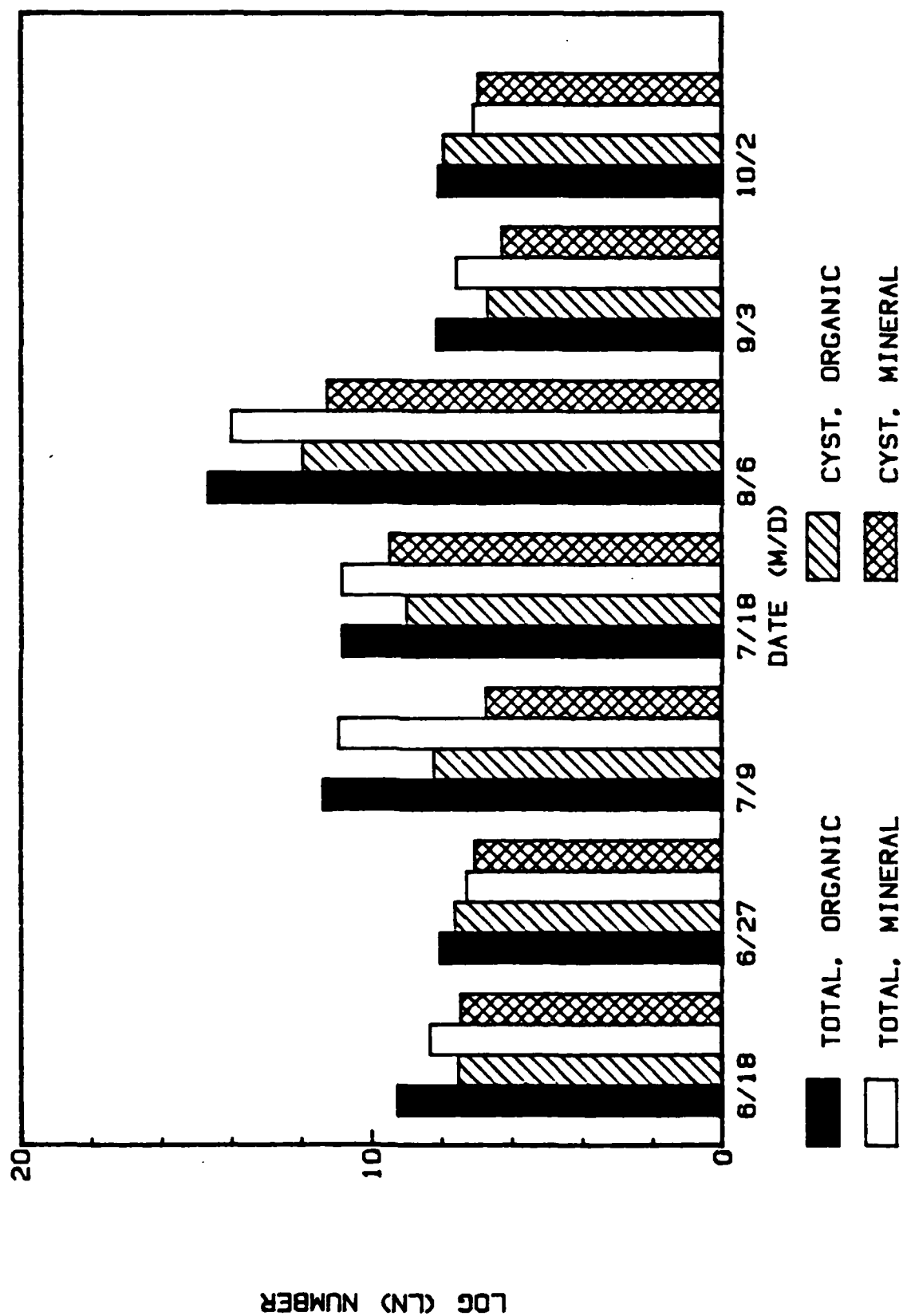


FIGURE 4. Average total number of amoebae at the 3 sites, ORGANIC HORIZON.

ORGANIC HORIZON (1984)

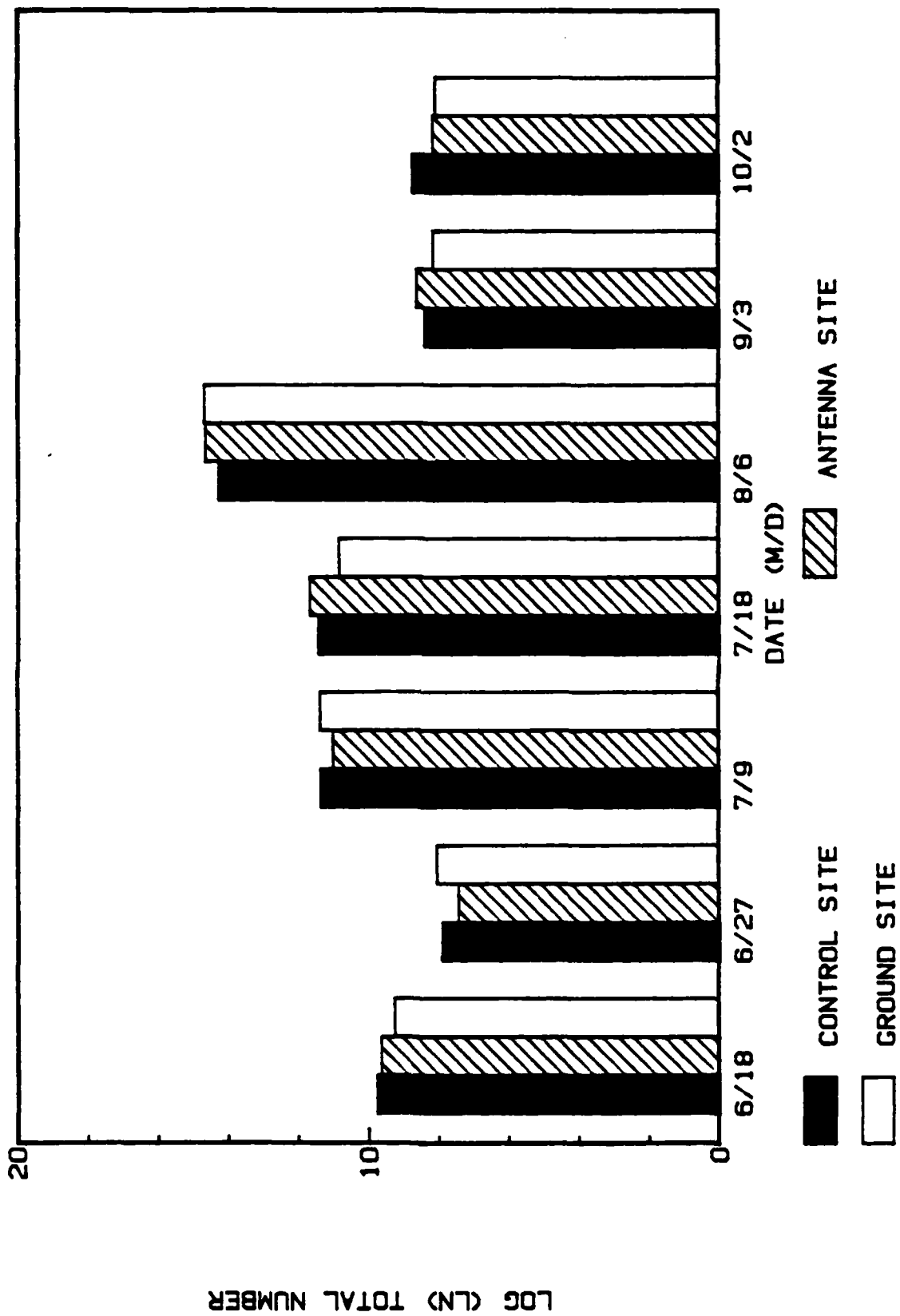


FIGURE 5. Average total number of amoebae at the 3 sites, MINERAL HORIZON.

MINERAL HORIZON (1984)

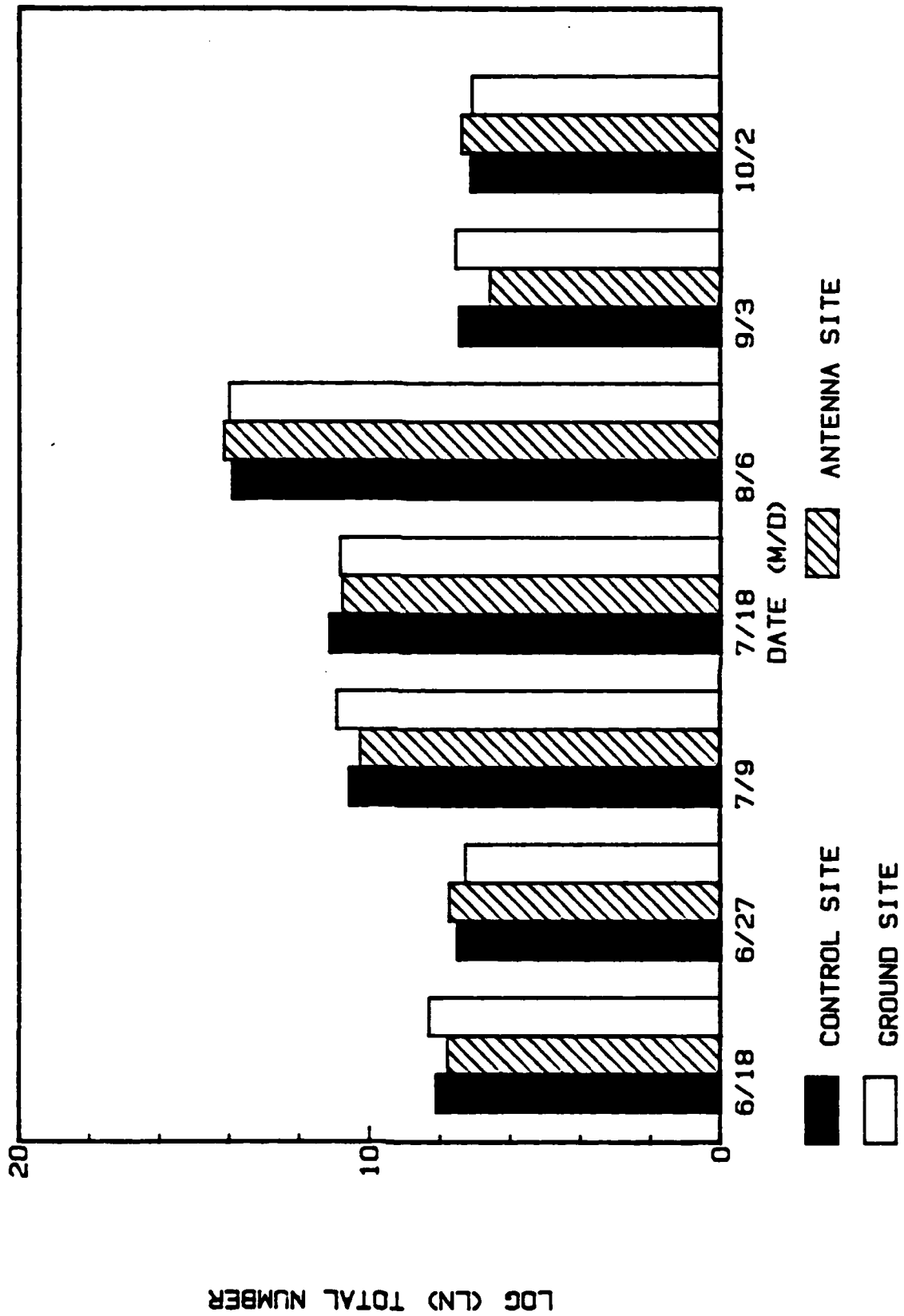


FIGURE 6. Calculated number of vegetative amoebae from Table 4 and 4A (by subtracting means) for the organic horizons; the 8/6 counts go off scale.

VEG.. ORGANIC (1984)

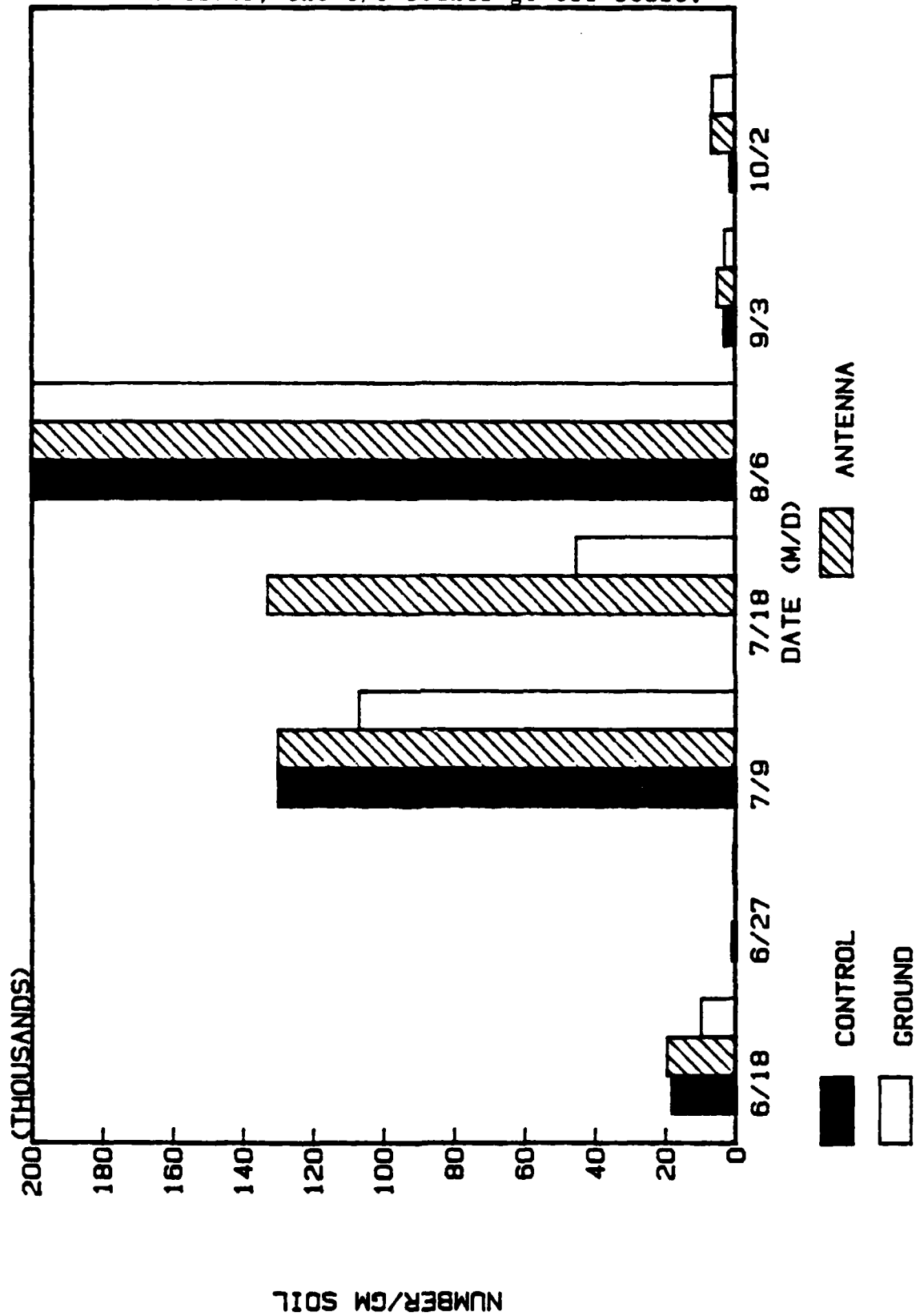


FIGURE 7. Calculated number of vegetative amoebae from Table 4 and 4A (by subtracting means) for the mineral horizons; the 8/6 counts go off scale.

VEG.. MINERAL (1984)

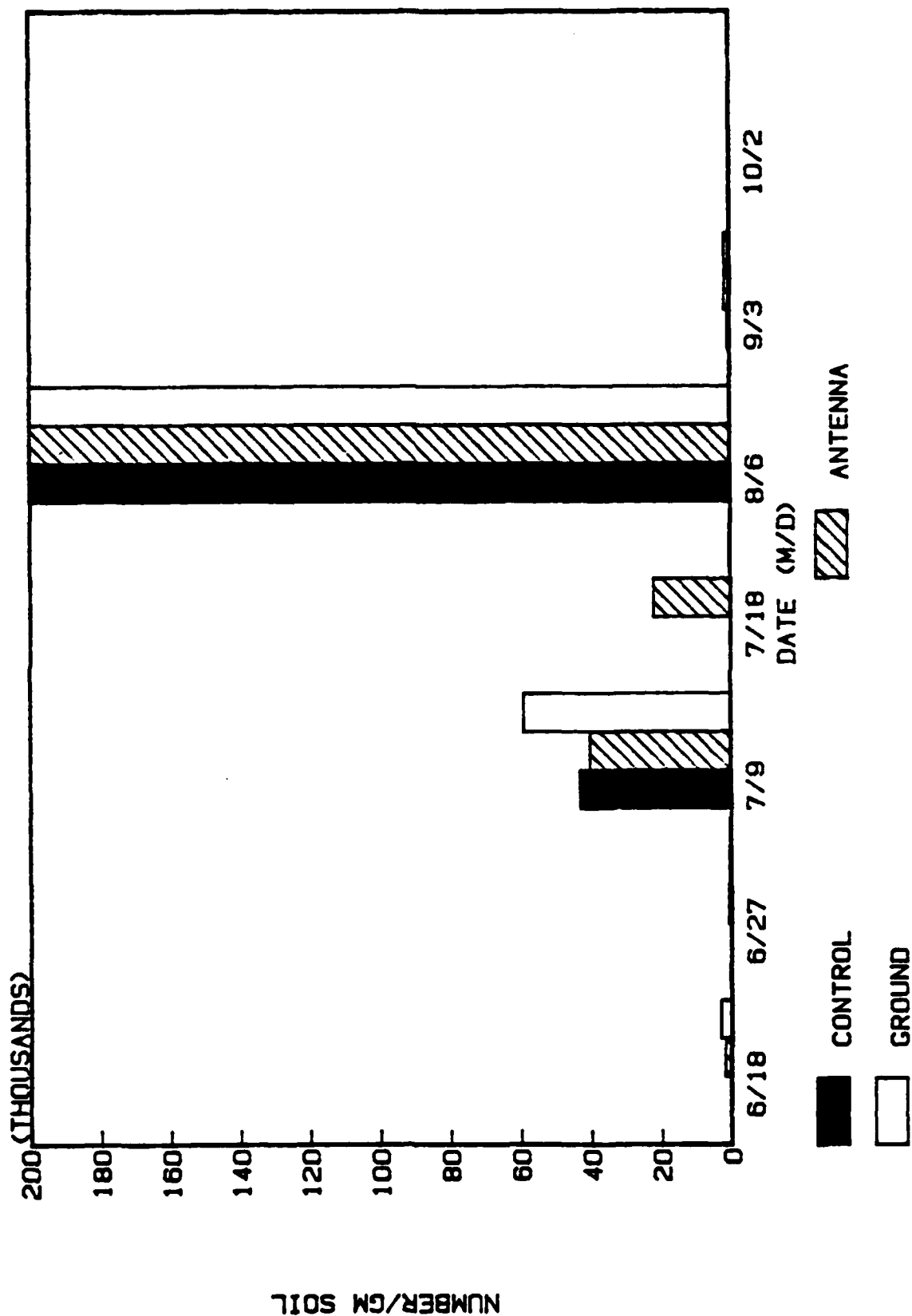


FIGURE 8. Plot of data used for regression calculations shown in Table 5.

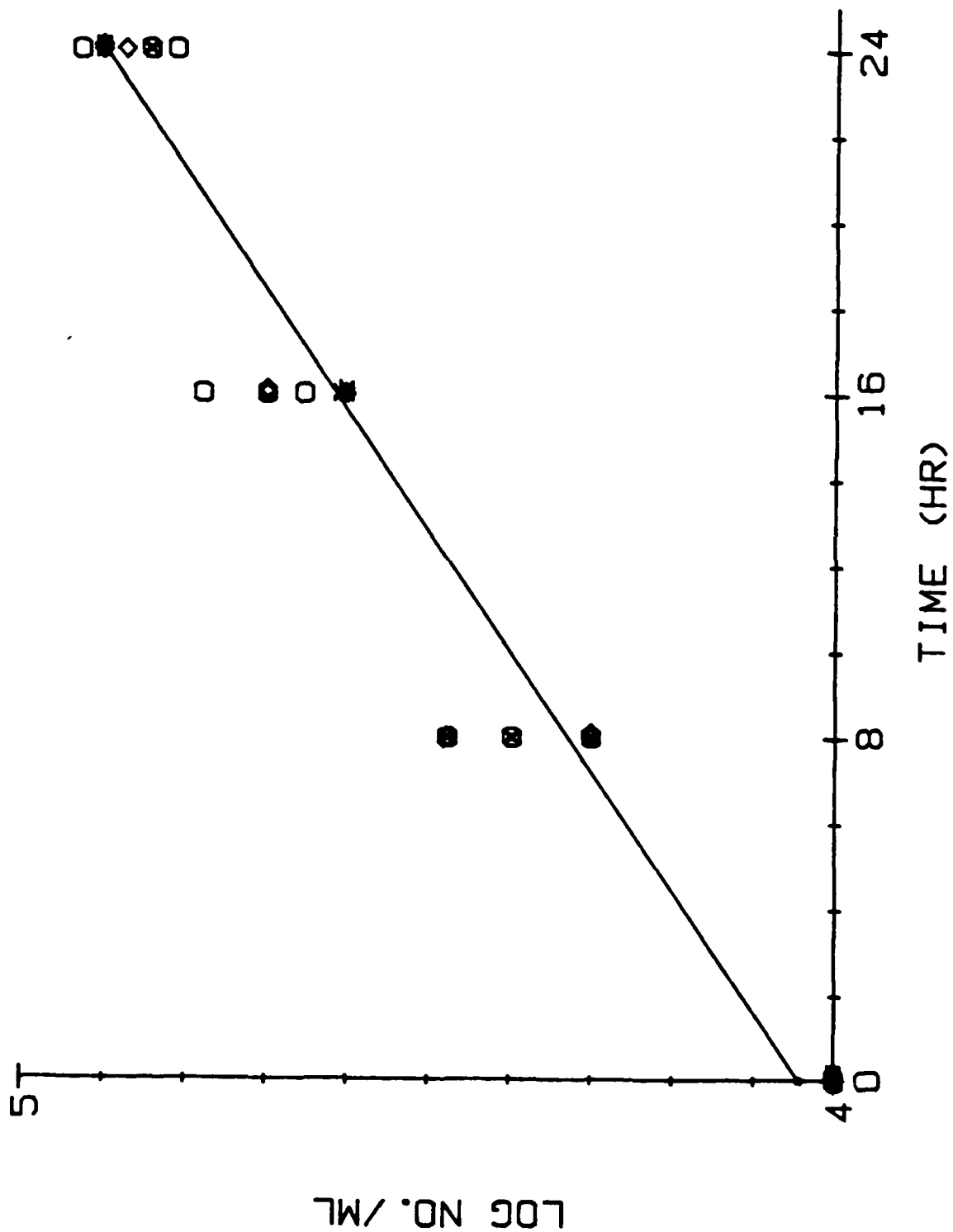


FIGURE 9. Control site, 4 hr. moisture recordings for the ORGANIC (upper plot) and MINERAL (lower plot). Arrows indicate sampling dates for soil counts.

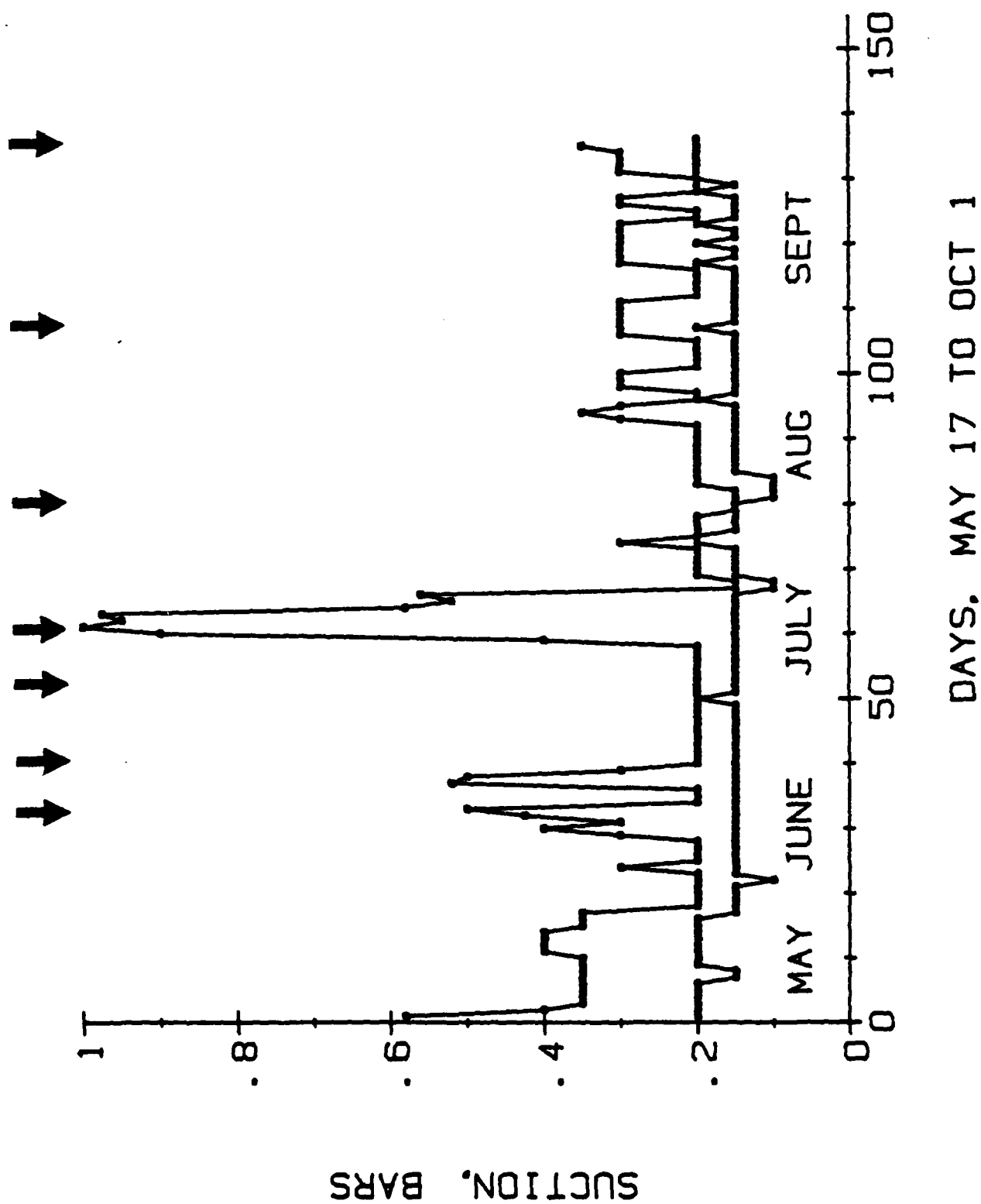


FIGURE 10. Moisture content of ORGANIC horizons for all sites.

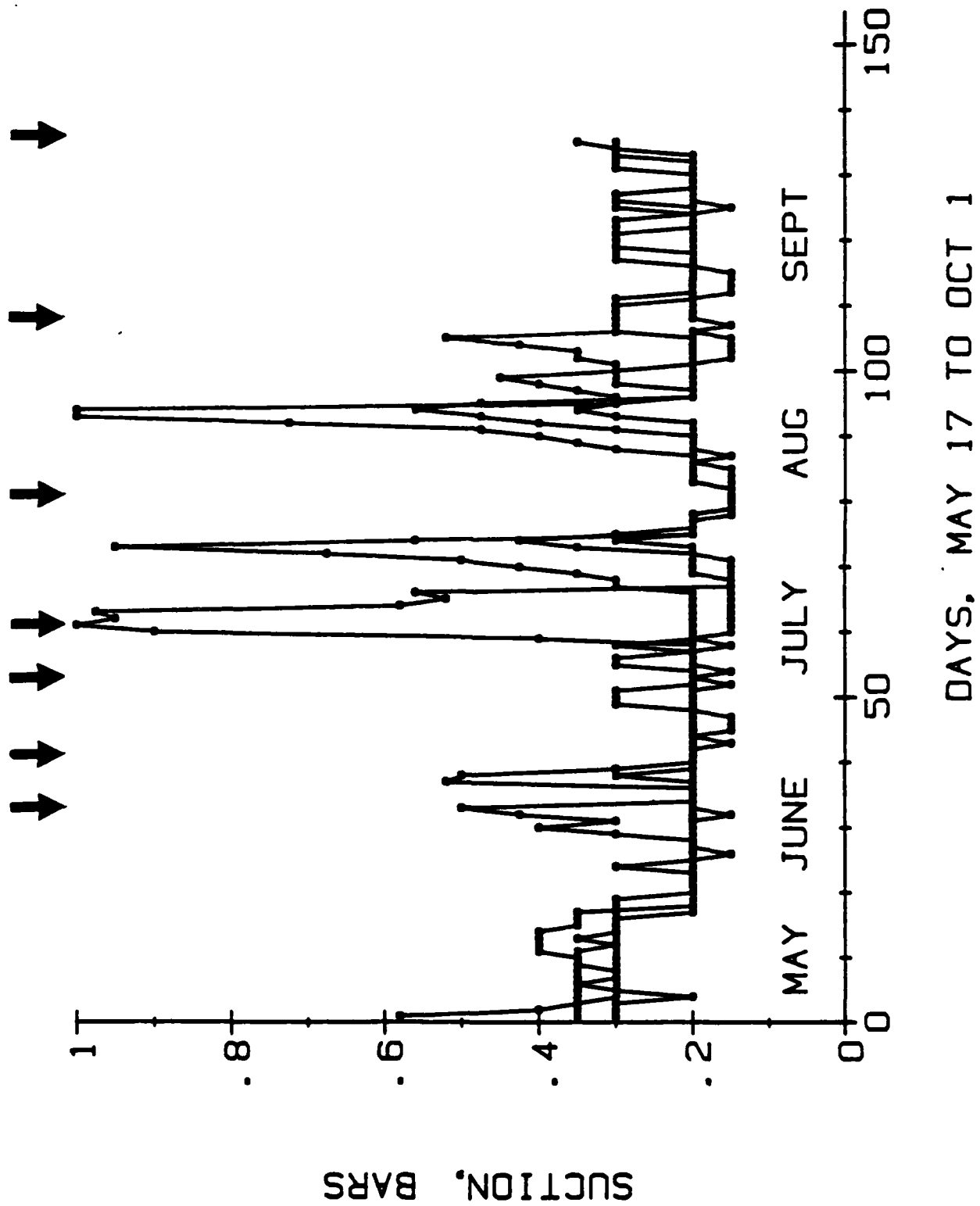
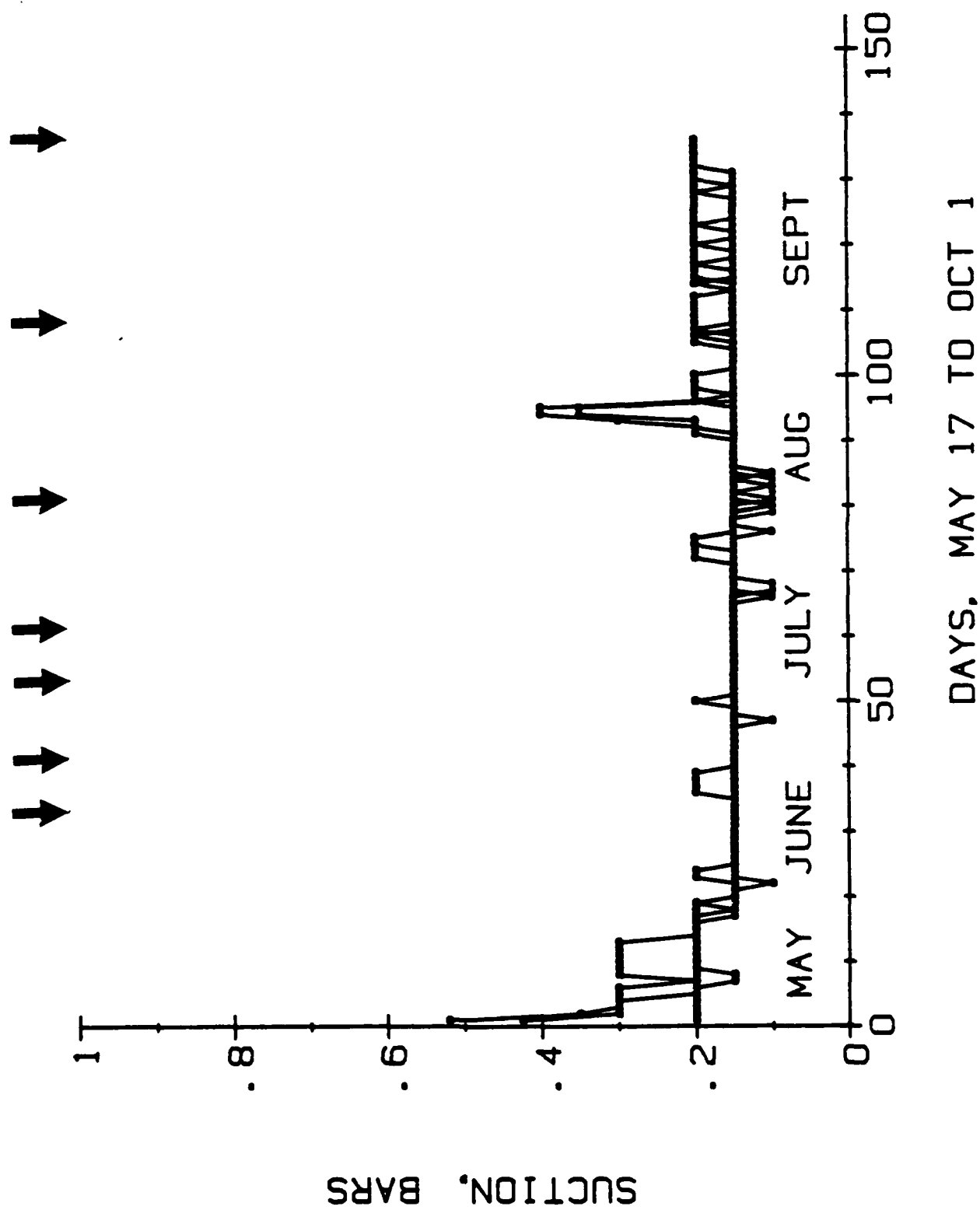


FIGURE 11. Moisture content of MINERAL horizon for all sites.



37.

FIGURE 12. Control site ORGANIC horizon temperature plotted every 4 hr. for 6 days.

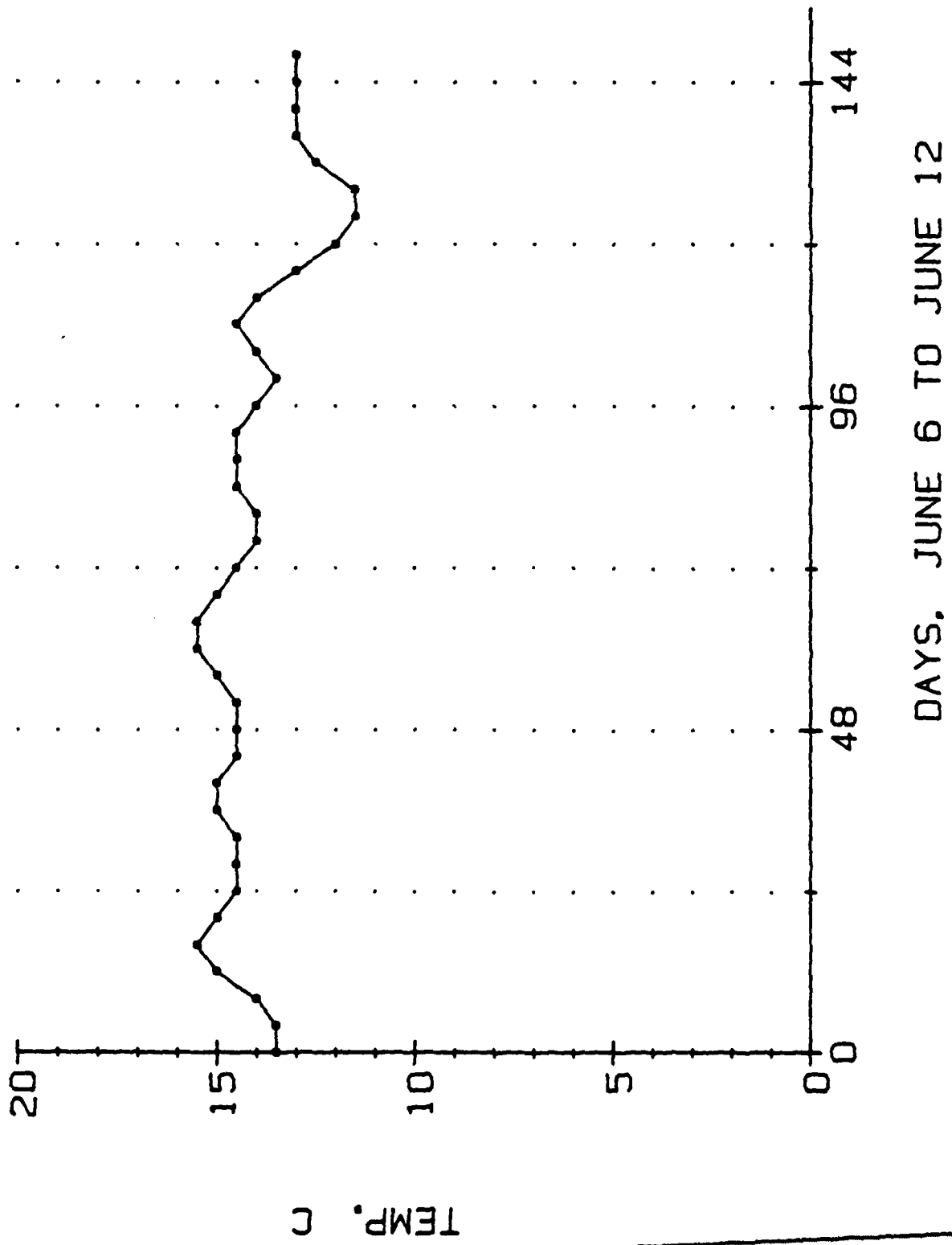


FIGURE 13. Control site 4 hr. temperature recording for the ORGANIC (upper plot) and MINERAL (lower plot).

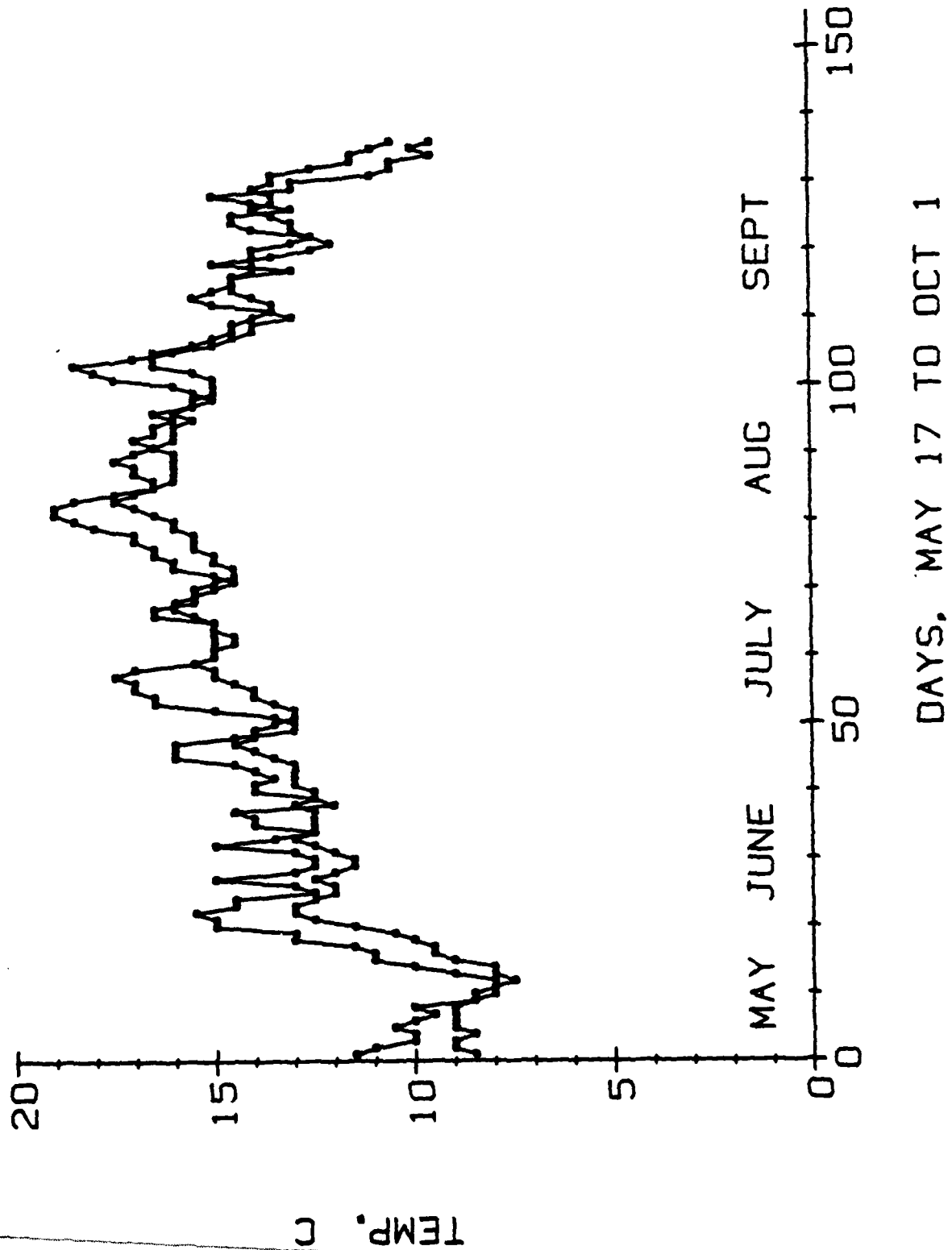


FIGURE 14. Temperature plot of ORGANIC horizons for all sites.

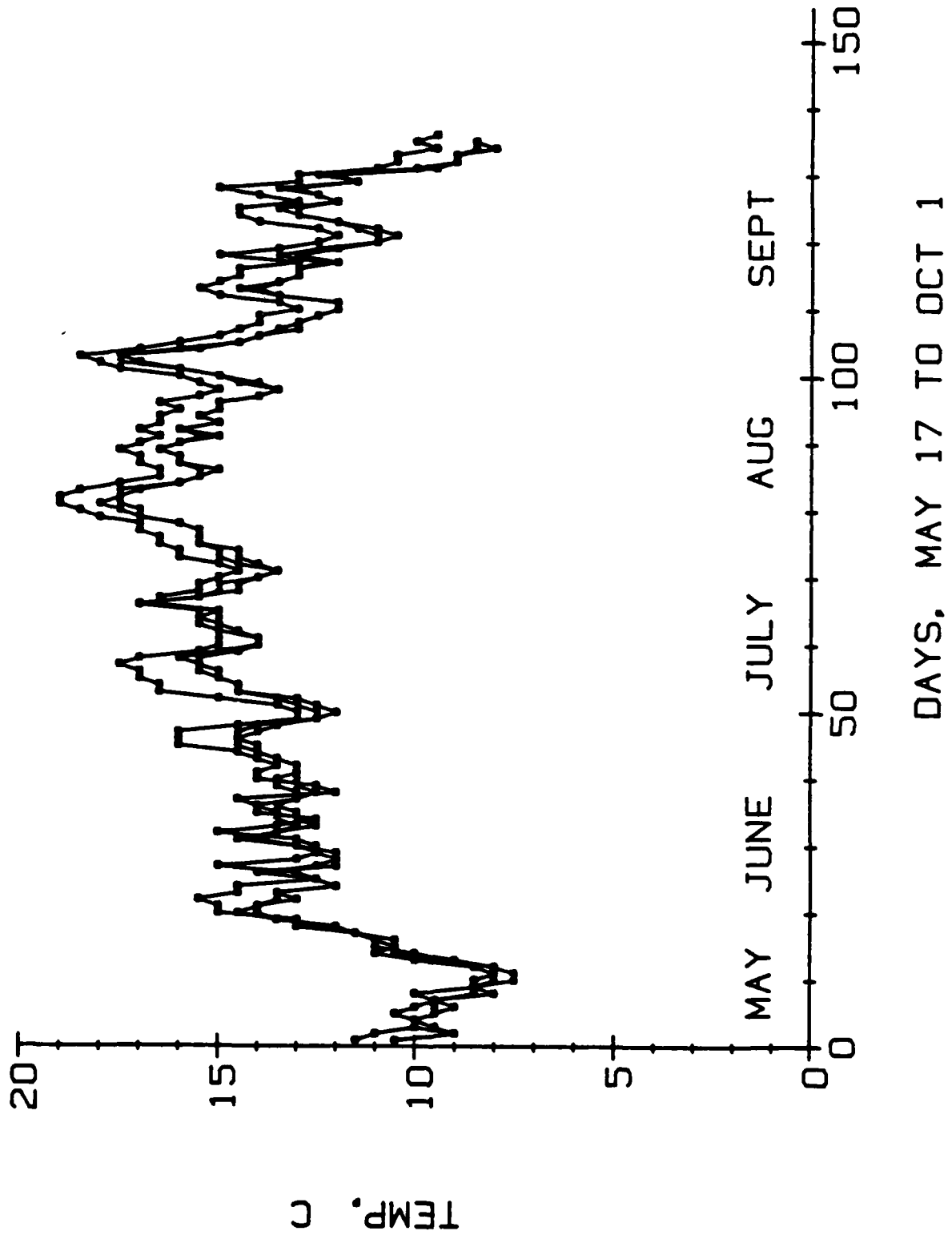
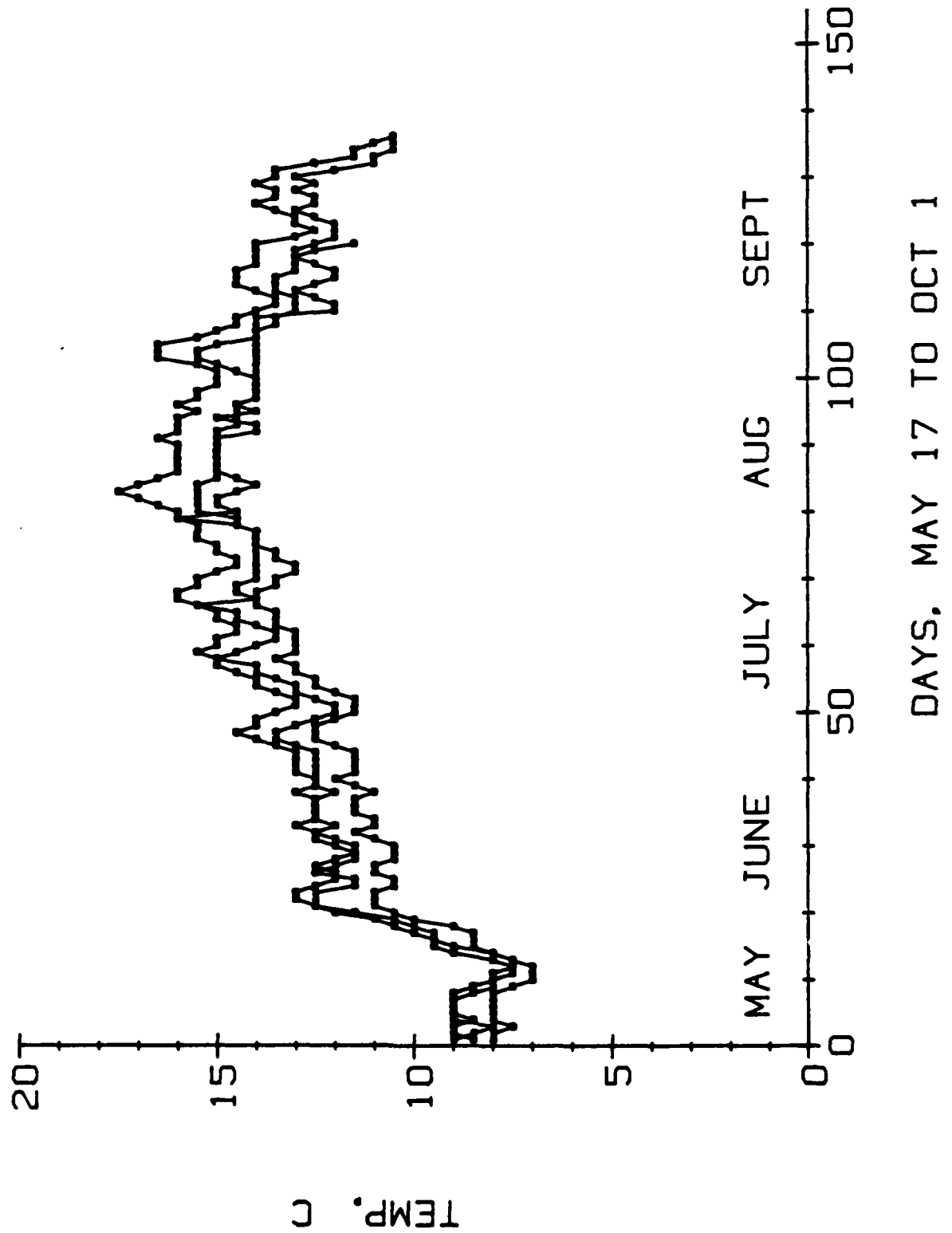


FIGURE 15. Temperature plot of MINERAL horizons for all sites.



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Subcontract No.
E06549-84-C-004

ELF Communications System Ecological Monitoring Program:

Soil and Litter Arthropoda and Earthworm Studies

Tasks 5.3. and 5.4.

1984

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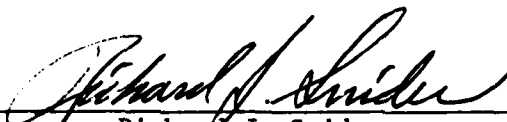
E06549-84-C-004

ELF Communications System Ecological Monitoring Program

Soil and Litter Arthropoda and Earthworm Studies

Tasks 5.3. and 5.4.

1984


Richard J. Snider

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Renate M. Snider

Co-Investigator

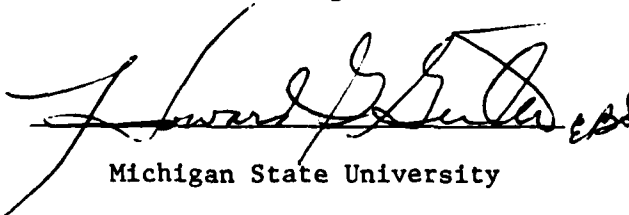

Michigan State University

TABLE OF CONTENTS

Abstract	1
Summary	2
Introduction	5
I. REVIEW AND PROGRESS	7
II. SITE CHARACTERISTICS	11
1. Site selection and design	11
2. Vegetation	12
3. Soils	16
4. Macro- and microclimate	17
III. SOIL- LITTER ARTHROPODA	23
1. Methods	23
2. Arthropod populations	26
IV. DIEL AND SEASONAL ACTIVITY	53
1. Methods	53
2. Surface-active arthropods	62
V. LUMBRICIDAE	75
1. Methods	75
2. Earthworm populations	78
VI. LITTER DECOMPOSITION	85
1. Methods	85
2. Litter input	88
3. Litter standing crops	88
4. Confined litter decomposition	92
5. Decomposition of trotline leaves, 1983-84	95
6. Leafpack study, 1984-85	96
VII. CONCLUSION	101
Appendix A: Ground cover in Test and Control	105
Appendix B: Manuscript 1	111
Appendix C: Manuscript 2	133
Appendix D: Manuscript 3	147

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ABSTRACT

Definitive sites (one Test, one Control) were surveyed and sampled from May 7 to October 15, 1984. Arthropod populations were monitored at two-week intervals. Preliminary data show that, while both sites harbor similar species spectra, relative abundances differed. Lumbricid associations, also sampled at two-week intervals, consisted of two surface-dwelling species shared between sites, and two soil-inhabiting species unique to each site. Each species in Test, however, has its equivalent (functionally and numerically) in Control. Site comparison based on vegetation, soil type and chemistry, and seasonal litterfall, was intensified in 1984. The sites were well matched with respect to these criteria. Leaf litter decomposition was monitored by two different methods (litterbags and unconfined leaves), mainly to assess the suitability of techniques. Possible site-specificity of breakdown rates became apparent in late fall. Experimental designs were then changed to accomodate known sources of variation, and continued breakdown studies were implemented. Techniques employed in various project elements were checked for potential sources of error, and their details have been finalized for use in the 1985 field season.

SUMMARY

From May 7 to October 15, 1984, Test and Control sites were sampled in accordance with the proposed schedule and replication. Resulting faunal material is not yet completely identified, so that analyses (other than preliminary site comparison) are pending. In addition to routine sampling, a number of potential sources of error were quantified. Results, conclusions and program changes are summarized below.

1. Site surveys were intensified in 1984. Soil textural profiles were found to be similar, fine sandy loam predominating in both sites, although the soils belong in different series. Macronutrients and pH did not differ significantly. Only extractable P, measured by a more sensitive method than in 1983, was significantly higher in Control.

Vegetation surveys were completed, including a mid-summer analysis of ground cover composition. Despite some differences in mean basal areas and species composition (e.g., absence of elm in Test), the sites are well matched.

2. Environmental monitoring included precipitation, and showed that rainfall events were essentially synchron in both sites. Sampling was expanded to include soil and litter moisture by weight loss after drying; litter moisture data promised to be an excellent explanatory tool for faunal fluctuations.

Automatic logging of soil temperature and moisture was plagued by equipment breakdowns. In view of the importance of these data, we decided to add sensing/recording devices of a different design in 1985, including a main and a backup system.

3. Arthropod populations, where identifications are available, were found to be very similar in species composition. Relative abundances of

members of major groups often differed between sites, and showed discrepant seasonal density fluctuations. Using mites as an example, a need for greater in-depth analysis of selected groups was shown. This re-direction of effort (from working with all material in semi-detail) will yield a more sensitive data base on the dynamics of selected populations.

4. Surface-active arthropods in Test and Control were similar in species composition, although some taxa, e.g. carabid beetles, were more diverse in Control. Pit-trapping techniques were further tested: results of a full-season barrier-trapping experiment led us to switch to the use of barriers beginning in 1985. Data showed that barriers magnified catches to a degree which varied with taxon ($>5x$ for carabids, $<2x$ for Collembola). Relative active density estimates must be interpreted with caution, therefore. Since routine trapping was performed simultaneously, we will be able to quantify these effects (pending species identification).

5. Lumbricidae were sampled at shorter (two-week) intervals in 1984, while fewer samples were taken per date. With the possible exception of cocoons, a reduced replication of 10 was shown to have little effect on coefficients of variation, i.e., on the accuracy of population estimates. Calculation of live worm biomass from preserved specimens was quantified for five species; all regressions were highly significant, and differed between species.

Lumbricid associations differed between sites in terms of species composition, although each species in Test has its ecological equivalent in Control. Of two litter-dwelling species shared between sites, Dendrobaena octaedra is prevalent in Control, Lumbricus rubellus in Test. One medium-sized soil-dwelling species (Aporrectodea turgida in Control, A. tuberculata in Test) dominates numerically in each site and contributes most of the biomass. Each site also harbors a moderately common deep-living

form. Because we accurately sample all life stages, potential effects of ELF on lumbricid biology should be reflected in population dynamics, detectable no matter the site-differences in species composition.

6. Leaf litter turnover: litter inputs to the forest floor were essentially equal in both sites, as was the response of trees to climatic conditions (gradual abscission in 1983, abrupt and late denudation in 1984). Of all decomposition criteria monitored, standing crop estimates proved the most variable. In 1985, additional samples will be taken at critical times of the season.

Litterbag studies were expanded to a large-mesh series of bags (5 mm) in addition to a replicate series of 1 mm bags. 1984 data (first year of decomposition) indicated a site-difference, Control retaining more of its initial weight than Test litter. Continued (second year) and replicate (second series of 1 mm bags) sampling will validate these results. In the case of arthropods extracted from litterbags, the value of genus- and species-level analyses is questionable because they are expected to be highly site-specific. We have tentatively decided that their densities, also likely to differ between sites, should still be obtained for all major groups present, since they represent a factor potentially affecting decomposition rates.

The trotline technique was proven unsuccessful because of the fragility of maple leaf petioles. Despite the resulting low sample replication, decomposition of unconfined leaves promised to be a good tool for assessing breakdown rates. Two major sources of variation were identified in 1984: position of leaves with respect to surrounding litter (vertical stratification) and leaf type (sun, shade). A revised experiment (leafpacks) was initiated in November 1984 which accomodates both variables as factors in future analysis of decomposition rates.

INTRODUCTION

The first samples to be taken in definitive sites were taken July 28, 1983. In 1984, sampling programs were conducted May through October. During this time, several accessory objectives were also implemented, dealing with: a) validation of techniques, and b) one-time surveys of general site characteristics, e.g. cataloging site vegetation.

We gave priority to obtaining data from these accessory objectives, and are just now re-focusing on processing the faunal material obtained during the field seasons. Because these samples are the first from definitive sites, initial progress was slow due to taxonomic difficulties. A complete "specimen base" necessary for a meaningful data base is therefore not at hand, but is being accumulated as rapidly as the growing Test/Control reference collection permits.

This 1984 annual report is to be read as an account of progress emphasizing results which help us plan future work. Conclusions with respect to methods, the suitability of objectives to overall project goals, and planned redistribution of effort toward certain objectives, are summarized in the last section.

I. REVIEW AND PROGRESS

The major objectives of our program, which monitors arthropods, lumbricids and selected breakdown processes in the soil-litter subsystem of deciduous forest, are listed in Figure 1. Their implementation was begun in late July 1983 and continued throughout 1984, following the experimental design proposed earlier and reiterated in section II of the present report.

In addition to routine sampling programs (for 1984 frequencies and sample totals consult Table 1), several minor study elements were carried out. They were mainly concerned with validating techniques and detecting potential sources of error, and detailed accounts of them may be found in pertinent subsequent sections. Here we simply identify them in order to complete the general account of 1984 activities:

a. Soil core samples: the efficiency of our heat extraction apparatus was checked in spring 1984, by sugar-floating samples after complete faunal extraction.

b. Pit-trapping: prompted by concern over low catches of some arthropod taxa, a full-season barrier-trapping experiment was performed on the usual weekly diel schedule, and supplemented by a short-term study on the effects of funnel inserts on catches.

c. Litterbags: selective invasion of litterbags by small lumbricids was documented by extracting "worm litterbags" concurrently with routine litterbag sampling. In addition, extraction of arthropods from intact litterbags was compared to extraction from litter after its removal from the bags.

d. Chilopod densities: geophilomorph population estimates have been based on specimens recovered during handsorting of lumbricid samples. The efficiency of the method was checked by heat-extracting and sugar-floating

humus squares taken next to worm samples.

e. A separate set of litter squares and soil cores were taken on each biweekly date for determination of water content, soil chemistry and litter standing crops. Resulting data aid interpretation of faunal data by supplementing the relatively unreliable readings from buried moisture sensors (gypsum blocks).

Table 1. Summary of 1984 sampling activities in Test and Control. First sampling date: May 7, 1984.

Category	N quadrats samp./site	Frequency	N samples total/site
Soil cores	20	biweekly	240
Litter 1/16 m ²	20	biweekly	240
Diel traps	20	weekly	880
Worms, 4 depths	10	biweekly	120
Litterbags	(8/date)	monthly	56

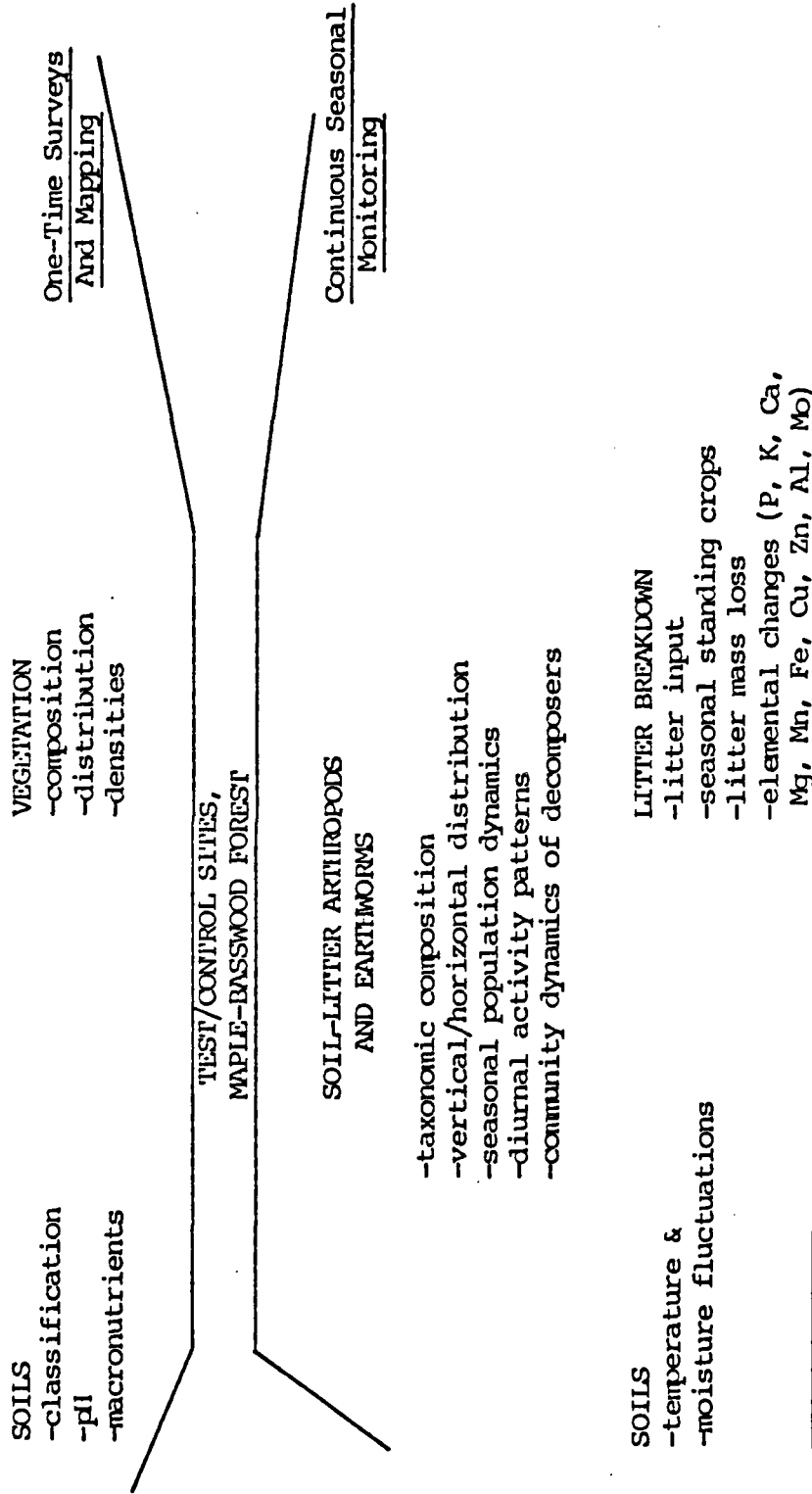


Fig. 1. Ecological monitoring objectives in Test and Control sites.
Implementation of major objectives was begun in late
July 1983. .

II. SITE CHARACTERISTICS

This section summarizes parameters (other than faunal/decomposition) used in site comparison. Included are vegetation data supplementing, and partly replacing, those reported in 1983: tree-shrub surveys of the Control site were repeated in 1984, after the grid quadrats had to be re-aligned with greater precision.

1. SITE SELECTION AND DESIGN

Two sites were chosen in 1983 in maple-dominated forest: a Test site adjacent to the future antenna corridor, and a Control site approximately 10.5 km distant from it. Levels of electromagnetic exposure, both present (measured) and future (estimated), were major selection criteria. They can be expressed in terms of field intensity ratios, as follows:

- 1) $\text{Test (ELF)} / \text{Control (ELF)} \geq 10$
- 2) $\text{Test (ELF)} / \text{Test (60)} \geq 10$
- 3) $\text{Test (ELF)} / \text{Control (60)} \geq 10$
- 4) $0.1 \leq \text{Test (60)} / \text{Control (60)} \leq 10$

where:

Test (ELF) = field intensity at the expected 76 Hz frequency of the ELF system at the Test site;

Control (60) = field intensity at a frequency of 60 Hz at the Control site; etc...

The sites formed an acceptable pair with respect to these criteria. Electromagnetic fields in the earth, in particular, fell well within the ranges postulated above (Table 2).

Table 2. EM field intensities and flux densities in Test and Control. NOTE: values for 76 Hz estimated according to distance from the antenna and expected operating frequency.

	SITE	
	TEST	CONTROL
Measured 60 Hz		
transv.el.field		
intens.,air, V/m	<0.001	<0.001
long.el.field		
intens.,earth, mV/m	0.11-0.27	0.018-0.063
magnetic flux		
density, mG	<0.001	0.001
Estimated 76 Hz		
transv.el.field		
intens.,air, V/m	0.1	<0.001
long.el.field		
intens.,earth, mV/m	57-65	4
magnetic flux		
density, mG	4-7.5	0.03

Each site was divided into a grid of 10 x 10 m quadrats separated by 1.5 m walkways. Twenty quadrats per site were designated for sampling, with one sample of a given kind taken per quadrat. Several were rejected because of their vegetational or topographic uniqueness. A few were left completely undisturbed for long-term photographic comparison with sampled quadrats.

2. VEGETATION

All trees were mapped by recording species, dbh (canopy: >12.7 cm; understory: 1.0-12.6 cm) and condition (live vs. standing dead). Shrubs, including young trees <1.0 cm dbh, were recorded if taller than 50 cm. Position within a quadrat according to x/y coordinates was mapped for all categories.

Ground cover abundance was estimated visually: each quadrat was divided into 4 (5x5 m) subplots, for a total of 100 per site. Both sites were surveyed by the same team of 2 people, who assigned each species an abundance value on an arbitrary scale of 0 to 3:

- 0 - rare
- 1 - occasional
- 2 - abundant
- 3 - very abundant

Included in the ground cover category were tree seedlings and shrubs < 50 cm tall. Jaccard's and Sorensen's indices of similarity were calculated to obtain a rough comparison of ground cover in the two sites.

Results:

For reference, common and scientific names of shrubs and trees present in Test and/or Control are listed in Table 3. Common names will be used in text and subsequent Tables. Importance values (Table 4), based on combined understory and canopy data show that the sites harbor similar stands. A major difference is the high value for elm in Control. While densities are essentially equal for the dominant, maple, and for basswood canopy, basal areas differ (Table 4): Test contains relatively larger trees of both species. Among minor stand elements, hornbeam is equally common in both sites' understory, but elm is virtually absent in Test (Table 5).

Table 3. List of tree and shrub species in Test and Control.

SPECIES	COMMON NAME
<u>Acer saccharum</u> Marsh	sugar maple
<u>Tilia americana</u> L.	basswood
<u>Ulmus americana</u> L.	elm
<u>Ostrya virginiana</u> (Mill) K. Koch	hornbeam
<u>Populus tremuloides</u> Michx	quaking aspen
<u>Betula lutea</u> Michx.f.	yellow birch
<u>Populus grandidentata</u> Michx	bigtooth aspen
<u>Dirca palustris</u> L.	leatherwood
<u>Hammamelis virginiana</u> L.	hazelnut
<u>Ribes</u> spp.	gooseberry
<u>Amelanchier canadensis</u> (L.) Medic	serviceberry
<u>Abies balsamea</u> (L.) Mill.	balsam fir
<u>Populus balsamifera</u> L.	balsam
<u>Prunus serotina</u> Erhr.	black cherry

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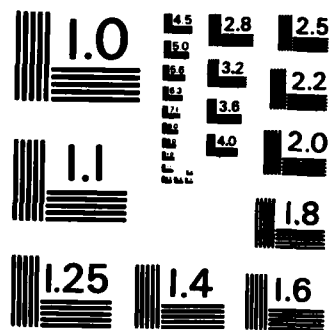
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MICROCOPY RESOLUTION TEST CHART
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Table 4. Importance values (relative density + relative dominance + relative frequency) of tree and understory species in Test and Control.

SPECIES	SITE	
	Test	Control
Maple	191.2	169.1
Basswood	71.9	62.8
Elm	2.7	26.9
Hornbeam	11.0	9.2
Poplar spp.	23.2	21.6
Yellow birch	-	8.3
Black cherry	-	1.5

Table 5. Canopy and understory in Test and Control: actual density and mean basal area of common species. Area surveyed: Test, 2400 m²; Control, 2500 m². NS = not significant; * = P < 0.05.

	Actual density		Mean basal area \pm SE		
	T	C	T	C	
Maple canopy	118	121	272 \pm 15.6	236 \pm 9.7	*
understory	350	381	36.2 \pm 1.6	36.5 \pm 1.7	NS
Basswood canopy	40	50	614 \pm 63.6	367 \pm 24.6	*
understory	6	20	89.1 \pm 12.7	60.5 \pm 6.3	*
Hornbeam canopy	1	1	(156)	(129)	
understory	12	11	44.6 \pm 15.5	66.6 \pm 13.5	NS
Yellow birch c.	-	3	-	550 \pm 98.0	
understory	-	2	-	92.7 \pm 6.3	
Poplar spp. c.	9	12	470 \pm 76.5	668 \pm 87.1	
Elm canopy	-	3	-	189 \pm 7.9	
understory	1	20	(7.8)	39.9 \pm 6.1	

Table 6. Number and mean basal area of standing dead in Test and Control.

	Mean dbh \pm SE, cm, (N)	
	TEST	CONTROL
Maple	183.4 \pm 48.8 (2)	(530.7) (1)
understory	15.6 \pm 1.4 (106)	10.2 \pm 0.7 (145)
Basswood	(145.2) (1)	(342.9) (1)
understory	-	53.3 \pm 6.6 (14)
Hornbeam	-	-
understory	44.2 \pm 9.8 (5)	29.5 \pm 6.8 (5)
Elm	-	341.5 \pm 181.0 (2)
understory	-	31.0 \pm 5.8 (17)

Standing dead consist mainly of maple understory in both sites. Control alone also contains small dead elm and basswood (Table 6).

Leatherwood dominates the shrub associations in both sites, followed by hornbeam (Table 7). Leatherwood densities are not significantly different ($P > 0.1$). The sites are most dissimilar with respect to hazelnut, present mainly in Test, and small poplars, relatively frequent in Control (Table 7).

Table 7. Shrub and seedling populations in Test and Control; frequency, in parentheses, = n quadrats in which a species occurs / total quadrats surveyed.

Species	Mean / 100 m ²	SE
	TEST	CONTROL
Leatherwood	3.8+1.1 (0.67)	9.0+1.3 (0.84)
Hornbeam	1.6+0.6 (0.50)	1.3+0.4 (0.48)
Hazelnut	2.7+1.4 (0.17)	0.1+0.1 (0.04)
Poplar spp.	0.1+0.1 (0.04)	3.3+1.1 (0.48)
Balsam fir	1/2400 m ²	2/2500 m ²
Gooseberry	2/2400 m ²	3/2500 m ²
Maple	3/2400 m ²	2/2500 m ²

Of 53 species of ground cover (or higher taxa where identification was not possible), 14 were unique to Test, while 4 were present only in Control. Nine shared taxa occurred in more than 50% of the subplots in both sites: Maianthemum canadense Desf., sedge spp., Ozmorhiza claytonii (Michaux) Clarke, Acer saccharum Marsh, Polygonatum commutatum (R. & S.) Dietr., Botrychium virginianum (L.) Sw., Taraxacum spp., Viola pubescens Ait., and Trillium grandiflorum (Michx.) Salisb. Details of relative abundances and frequencies for all taxa encountered can be found in Appendix A.

Jaccard's and Sorensen's indices of similarity were relatively high (70.6 and 81.8 % respectively), but Sorensen's index gives greater weight to species common to both sites rather than to those unique to each.

Ground cover surveys, so far based on mid- to late-summer data, will be supplemented by brief surveys in spring of 1985, in order to catalog vernal species missed in 1984.

3. SOILS

The area containing the Control site is mapped as Demontreville loamy fine sand (arenic Eutroboralfs, loamy, mixed). Test soils are classified as Pemene fine sandy loams (eutric Glossoboralfs, coarse-loamy, mixed, frigid). Table 8 gives a summary of profiles in each site, based on 2 to 3 auger samples; depths of layers represent averages based on these few samples.

Although the soils are in different soil series, the profiles are texturally similar, with fine sandy loam predominant (Table 8). Below approximately 50 cm, both soils consist mainly of loamy sand.

Table 8. Major profile characteristics of Test and Control soils to a depth of approximately 50 cm.

Horizon	Depth (cm) and texture	
	TEST	CONTROL
A	0-11 fine sandy loam	0-14 fine sandy loam/ loamy fine sand
B2hir	-	14-20 fine sandy loam
B2ir	11-38 sandy loam/ fine sandy loam	20-36 fine sandy loam
	38-51 fine sandy loam to gravelly loamy fine sand	36-57 loamy sand/ sandy loam

1983 soil chemistry data were validated by sampling 10 quadrats per site on 4 dates in 1984. Unlike the previous year, P was determined by sodium bicarbonate extraction, which is more accurate than Bray extraction in

Ca-rich soils.

Average values for two depths are given in Table 9 (summed over 4 dates, i.e. 40 samples/depth/site). Only P was significantly higher in the upper B horizon in Control, where a B2hir layer is present (Table 8). The sites were well matched with respect to all other variables (Table 9).

Table 9. Macronutrients (kg/ha) and pH in the A horizon and approx. 5-10 cm below A (means \pm SE). * = significant at $P \leq 0.05$.

	A HORIZON		UPPER B HORIZON	
	TEST	CONTROL	TEST	CONTROL
pH	5.95 \pm 0.06	5.85 \pm 0.06	5.91 \pm 0.07	5.81 \pm 0.05
P	13.0 \pm 4.81	12.1 \pm 2.79	12.5 \pm 1.63 *	17.8 \pm 1.63 *
K	117.1 \pm 5.68	104.8 \pm 4.86	74.4 \pm 3.43	71.5 \pm 3.26
Ca	2928 \pm 129.8	3120 \pm 152.5	1778 \pm 149.8	1542 \pm 103.5
Mg	227.0 \pm 12.66	245.6 \pm 11.99	131.8 \pm 8.25	133.2 \pm 7.11

4. MACRO- AND MICROCLIMATE

The area overall has a temperate continental climate of the cool summer type (30-year average temperatures for July: approx. 26 C max, 3 C min). Annual normal precipitation is 76 cm, with snowfall occurring from September to May.

In 1984, the following site-specific measurements were taken:

i. Precipitation:

Recorded late May through mid-October (non-recording rain gauges): minor local differences between Test and Control existed, but major rainfall events showed synchrony (Fig. 2). Total precipitation during summer and fall equalled 40.9 cm in Test and 43.8 cm in Control.

ii. Litter moisture:

On each sampling date, a 1/16 m² area of leaf litter was removed, adjacent to two other litter squares (for lumbricid and arthropod extraction). Soil clumps and woody matter were discarded quickly before

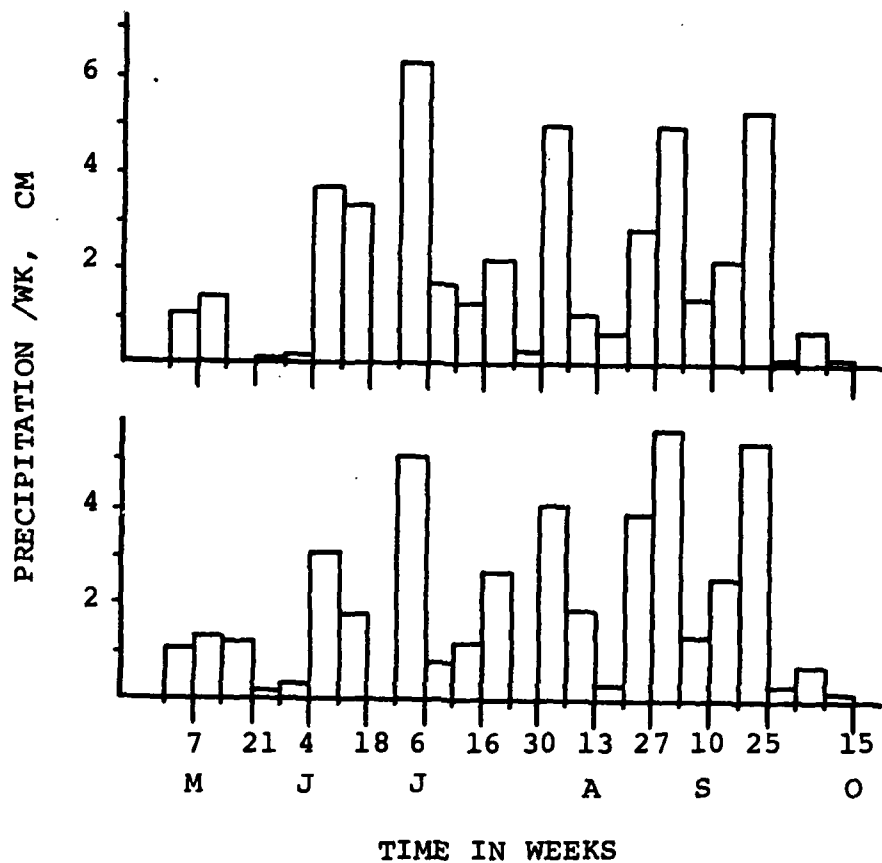


Fig. 2. Precipitation per week, 1984, in Test and Control; dates, May 7 through October 15, are sampling dates.

bagging the litter. Moisture, in % of dry weight, was determined by weight loss after oven drying.

From late May to early August, litter in both sites contained less than 50% water, with the exception of July 6 (Fig. 3): moistures differed significantly on that date because of a locally restricted light rain in Control. In early, May moisture was high due to some precipitation (Fig. 2), and to melting snow which had fallen a few days prior to sampling. In late summer and fall, heavy rains kept litter moisture at 100% or higher. Among three significantly different means, only one (July 6) was likely to be of biological significance.

Table 10. Correlation between percent moisture (M) and dry weight (W) of leaf litter in Test and Control. Data from both sites lumped, except those for July 6 (moistures differed) and September 10 (Control samples destroyed).

Date		Test	Control	r	P
5/7/84	M	134.8+7.4	148.7+9.9	-0.029	NS
	W	16.8+1.6	21.8+1.5		
5/21	M	7.7+0.6	11.3+2.0	0.602	0.001
	W	16.4+1.5	18.6+1.8		
6/4	M	11.0+1.3	10.4+1.2	0.779	0.001
	W	17.9+2.4	22.7+2.5		
6/18	M	41.1+9.4	42.5+3.9	0.802	0.001
	W	22.2+3.1	21.1+2.2		
7/6	M	42.3+2.5	117.6+5.5	0.311(T)	NS
	W	16.5+1.7	19.5+2.1	-0.090(C)	NS
7/16	M	30.5+3.3	38.0+5.3	0.8225	0.001
	W	13.5+1.8	21.8+2.7		
7/30	M	15.5+4.6	11.1+0.9	0.640	0.001
	W	11.5+2.4	18.9+2.7		
8/13	M	17.7+1.8	29.0+3.6	0.469	0.01
	W	14.0+2.4	18.8+2.0		
8/27	M	95.8+9.2	103.5+6.4	0.404	0.01
	W	15.4+2.9	21.6+2.7		
9/10	M	88.0+8.8	-	0.642	0.01
	W	8.6+1.2	-		
9/25	M	176.3+4.1	186.6+3.4	-0.022	NS
	W	13.5+1.6	10.9+0.9		
10/15	M	92.8+3.4	100.7+5.8	-0.250	NS
	W	22.3+1.3	18.7+1.1		

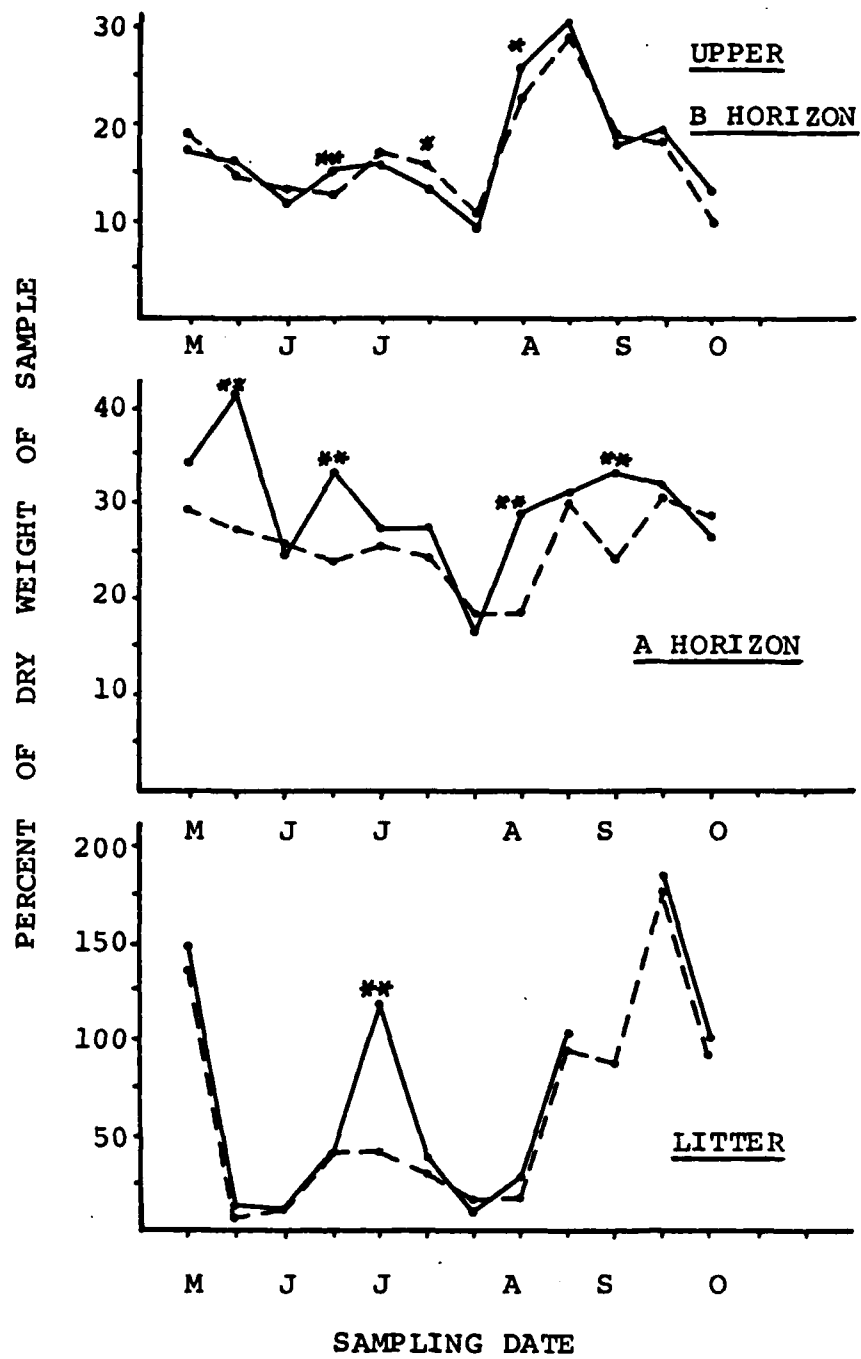


Fig. 3. Moisture, in percent of dry weight, in litter, A and upper B horizon. * = 0.05, ** = 0.01 level of significance.

On any one date, litter thickness at the sampling location was the major source of variation in water content, an effect tested by correlating dry weight and % moisture on each date. During dry periods, correlations were highly significant (Table 10): during wet periods, as expected, all samples were moist irrespective of litter thickness.

All three 1/16 m² samples (moisture, arthropods, worms) were taken from an area with approximately equal litter cover. Moisture and standing crop data can therefore be used as independent factors in faunal analyses. Direct use of faunal litter samples is, unfortunately, not feasible; arthropod samples must be manipulated as little as possible, and lumbricid samples are subjected to formalin extraction, so that neither can easily be used for moisture and mass determination.

iii. Soil moisture:

Continuous readings from buried gypsum blocks to data storage modules (Datapod DP 222, Omnidata International) have not yet been summarized and interpreted. A preliminary assessment in early 1984 indicated, however, that they were not overly reliable. We therefore retrieved, on each sampling date, approx. 100 ml of humus (A) and soil (upper B, 0-10 cm below A), and determined water content as % of air-dry weight.

The upper B horizon experienced synchronous moisture fluctuations in both sites. The A horizon, generally moister in Control than in Test, showed pronounced variability, although seasonal trends approximated those measured in litter and B layers (Fig. 3).

Where average moisture in the upper B differed at the 0.01 or 0.05 level (Fig. 3), the differences were probably not of biological significance. The discrepant averages obtained for the A layer may have been, to an unknown extent, due to human error; changes in sampling protocol have been made to

increase accuracy.

iv. Soil temperature:

Permanently installed sensors at depths of approximately 2, 10 and 20 cm were connected to data storage modules and their outputs were logged at 2 hour intervals. Except during occasional equipment malfunction, continuous records were obtained from late April to late September. The voluminous data have not yet been summarized. When they are, in terms of daily means and maxima/minima, we will be able to: a) compare sites (Test, unlike Control, has a slight south-easterly exposure); and b) use temperature data as independent factors in analyses of faunal data.

III. SOIL-LITTER ARTHROPODA

The bulk of quantitative data on arthropods are not yet formatted for publication. Preliminary Test-Control comparisons with respect to general faunal composition and selected groups are possible based on late-season 1983 data and some 1984 sampling results.

1. METHODS

i. Heat extraction efficiency:

A total of 32 soil cores, taken May 21, 1984, were floated in a saturated sugar solution after routine heat-extraction. Arthropods contained in the supernatant organic debris were then identified and counted.

In Table 11, results are shown for taxa of which ≥ 5 specimens were extracted. Surprisingly, the major conclusion to be drawn was that extraction efficiency differed with site, Control samples generally yielding a smaller percentage of total animals than Test (Table 11).

Table 11. Efficiency of heat extraction, in percent, for arthropods in Test and Control (soil core samples, May 1984).

	TEST		CONTROL	
	N	% extr	N	% extr
Pseudoscorpiones	-	-	7	100.0
Aranei	8	100.0	-	-
Acari	464	86.9	417	76.9
Chilopoda	-	-	7	70.0
Collembola total	573	85.4	297	75.2
Onychiuridae	402	84.6	149	61.6
Isotomidae	95	86.4	126	97.7
Entomobryidae	41	91.1	8	100.0
Sminthuridae	32	86.5	-	-
Coleoptera	8	80.0	-	-
larvae	14	58.3	5	33.3
Diptera, wingless	58	98.3	28	93.3
larvae	30	55.6	23	52.3
Diplura	8	100.0	-	-

We cannot trust these data. We plan again to check extraction efficiencies twice in 1985, to obtain valid correction factors for density estimates. The major source of error in present data stemmed, we believe, from sorting errors: dessicated animals and exuviae can be difficult to distinguish.

ii. Sorting efficiency: Chilopoda:

Geophilomorphs are relatively abundant soil-dwelling predators in both Test and Control. Their densities are estimated from specimens obtained as a by-product of handsorting 1/16 m² samples for earthworms. Some observations in 1984 indicated, however, that efficiency of geophilomorph recovery may vary with individual experience.

In October 1984 we took 10 (1/16 m²) humus samples in Control, adjacent to the block samples taken for lumbricids. They were quartered to fit available heat extraction funnels, and sugar-floated after extraction. In mid-October, we thus obtained geophilomorphs by: handsorting (Oct 15 earthworm samples); heat extraction; and floatation of extracted samples.

Results showed that :

a) extraction efficiency for A horizon blocks was 80.2% for all geophilomorphs (79.2% for Strigamia chionophila Wood, 82.8% for Taiyuna opita Chamberlin, total N = 101).

b) handsorting (which was, in part, performed by newly trained personnel) was about half as efficient as heat extraction (Table 12).

c) Small size classes were most likely to be overlooked: in S. chionophila, for example, classified according to total number of coxal pores on their ultimate legs, small individuals with 2 pores were obtained most frequently by heat extraction (Fig. 4).

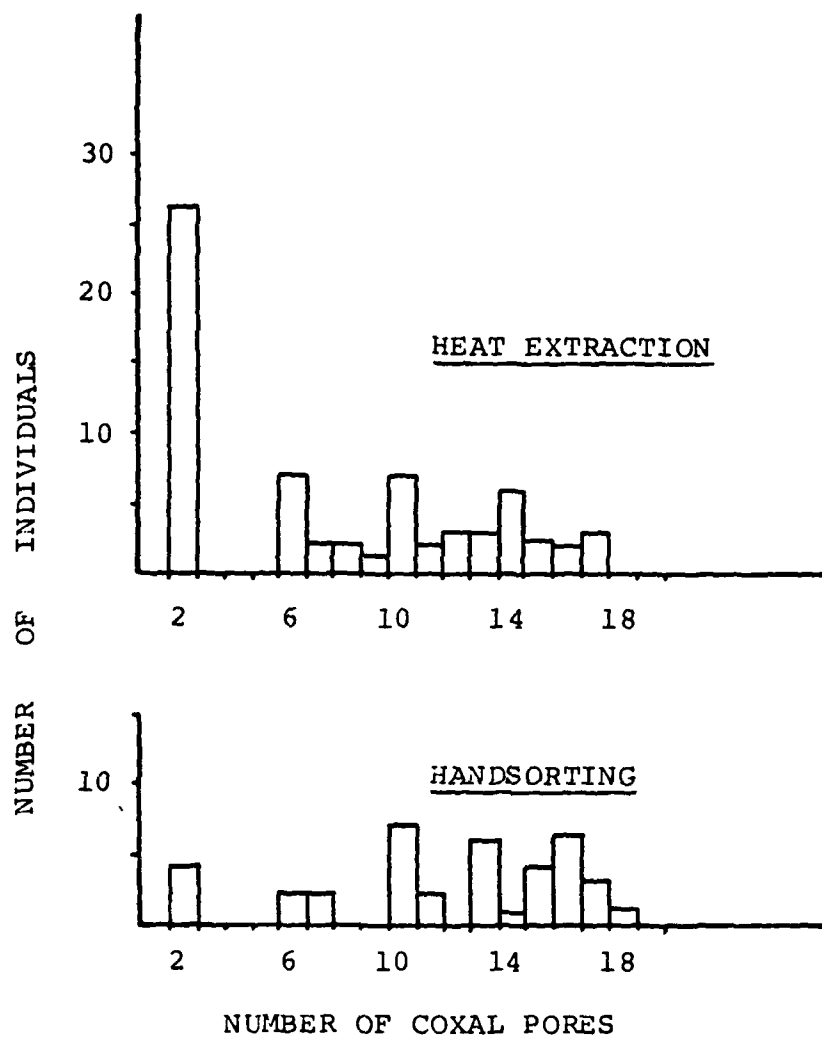


Fig. 4. Frequency distribution of size classes of S. chionophila, heat-extracted vs. handsorted: number of coxal pores increases with size.

Table 12. Mean geophilomorphs per sample obtained by handsorting (HS) and heat extraction (EX), and estimated densities/m² derived from each.

	<u>S. chionophila</u>		<u>T. opita</u>		Total	
	HS	EX	HS	EX	HS	EX
mean	3.5	5.7	0.8	2.4	4.3	8.1
SE	1.0	1.9	0.3	0.9	1.1	2.2
N/m ²	56.0	91.2	12.8	38.4	68.8	129.6
SE	16.4	30.0	5.2	13.7	17.6	34.9

Data from lumbricid samples obviously have to be interpreted carefully. Because we obtain large numbers of individuals by this technique, we plan to re-train personnel and continue sampling geophilomorphs this way. This group of predators is abundant enough to furnish a species-specific data base for both sites. Three or four times per season, however, heat-extraction of 1/16 m² A horizon samples will supplement and validate data obtained by handsorting.

2. ARTHROPOD POPULATIONS

1. Densities in litter and soil:

Total arthropod densities differed considerably with site (Fig. 5). Litter-dwellers in particular showed a numerical increase in late summer in Control, but not in Test.

Especially in leaf litter, mites (Fig. 6) were major contributors to these differences, outweighing Collembola, which tended to be more abundant in Test (Fig. 7). Aside from Acari and Collembola, larval Diptera (Fig. 8) and Coleoptera (Fig. 9), both principally soil-dwelling, contributed more to total arthropod densities than any other taxon.

Among non-insect predators, spiders (Fig. 10) reached surprising densities of 750/m² in Control soil in late summer. All spiders extracted

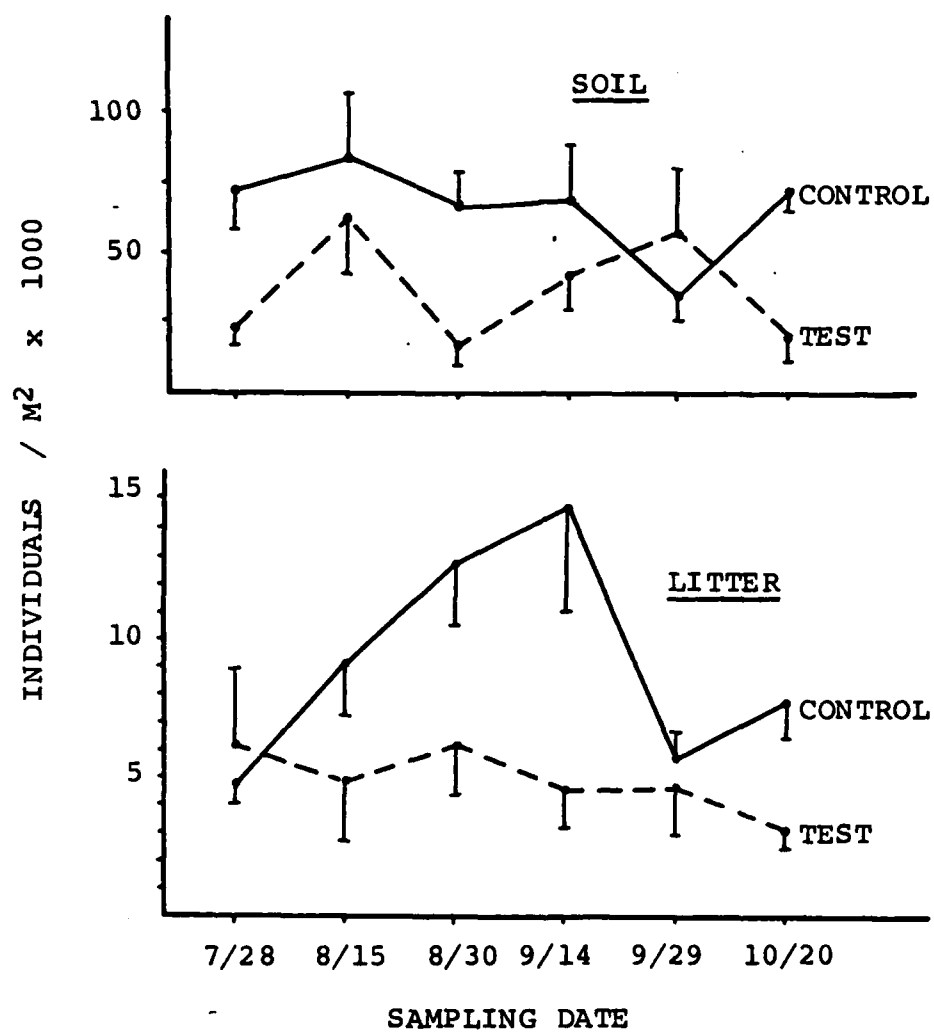


Fig. 5. Total arthropod densities/m² in Test and Control litter and soil, 1983, \pm SE.

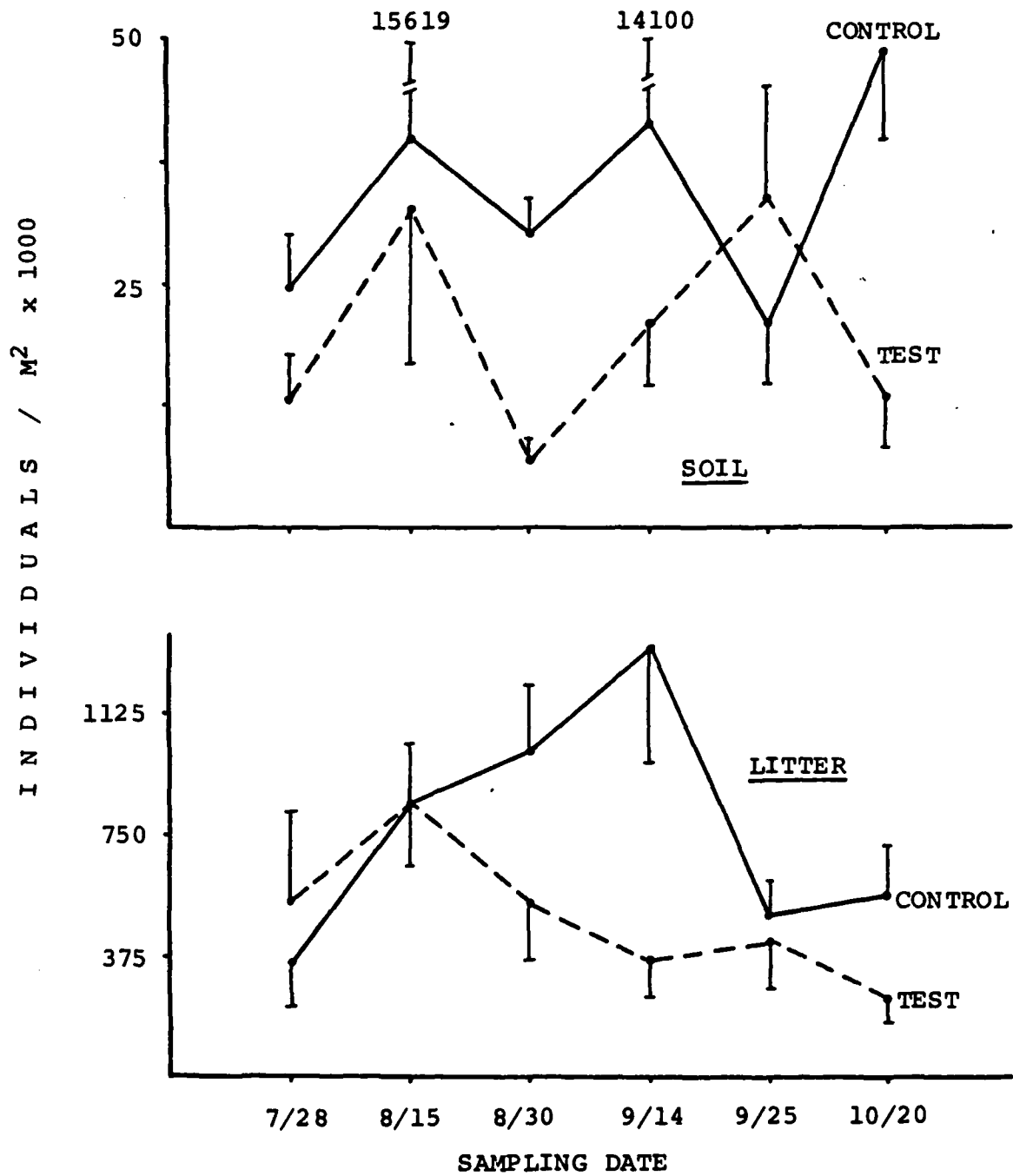


Fig. 6. Acari in litter and soil, 1983: estimated density/m² \pm SE.

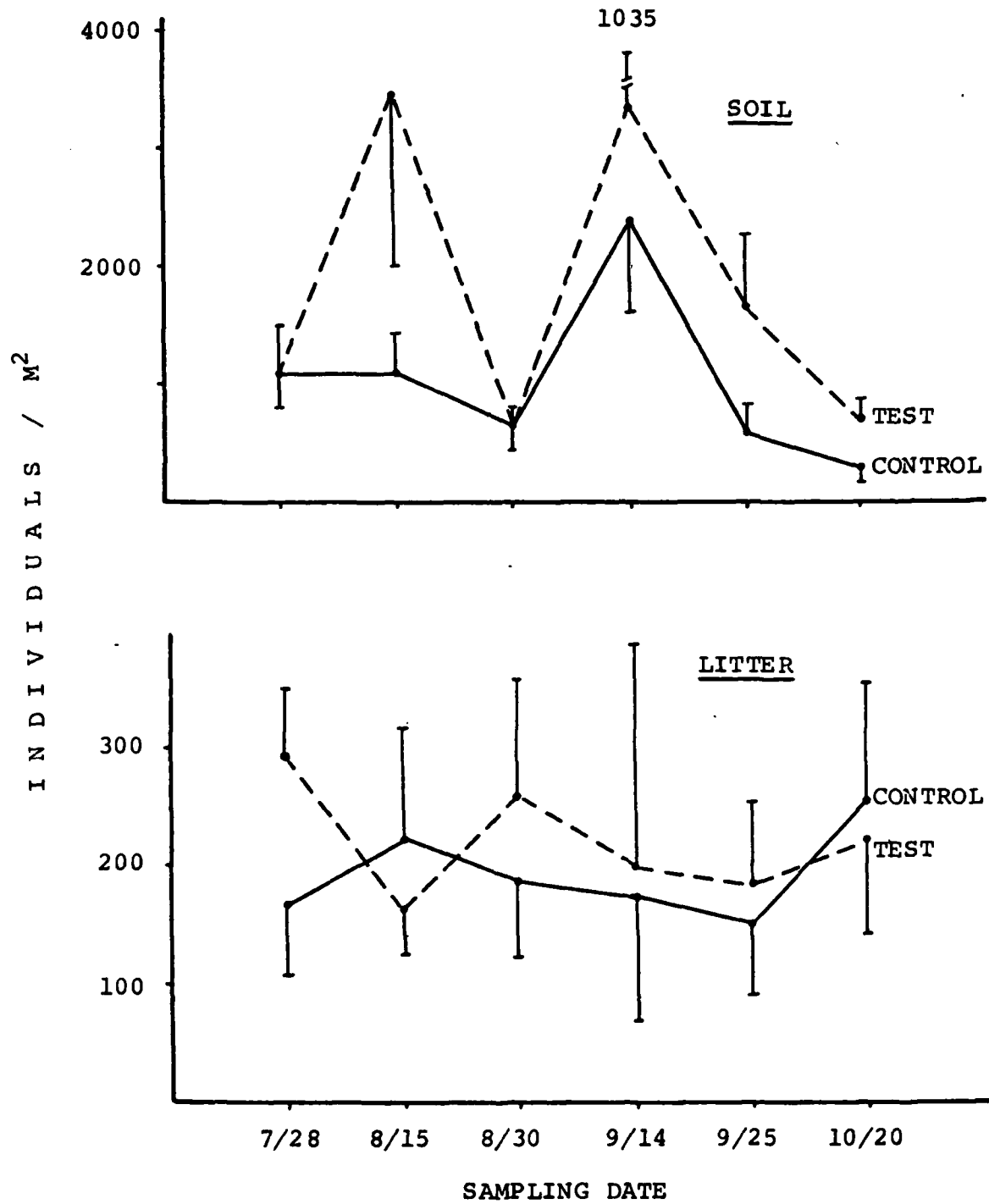


Fig. 7. Collembola in litter and soil, 1983, density/m² \pm SE.

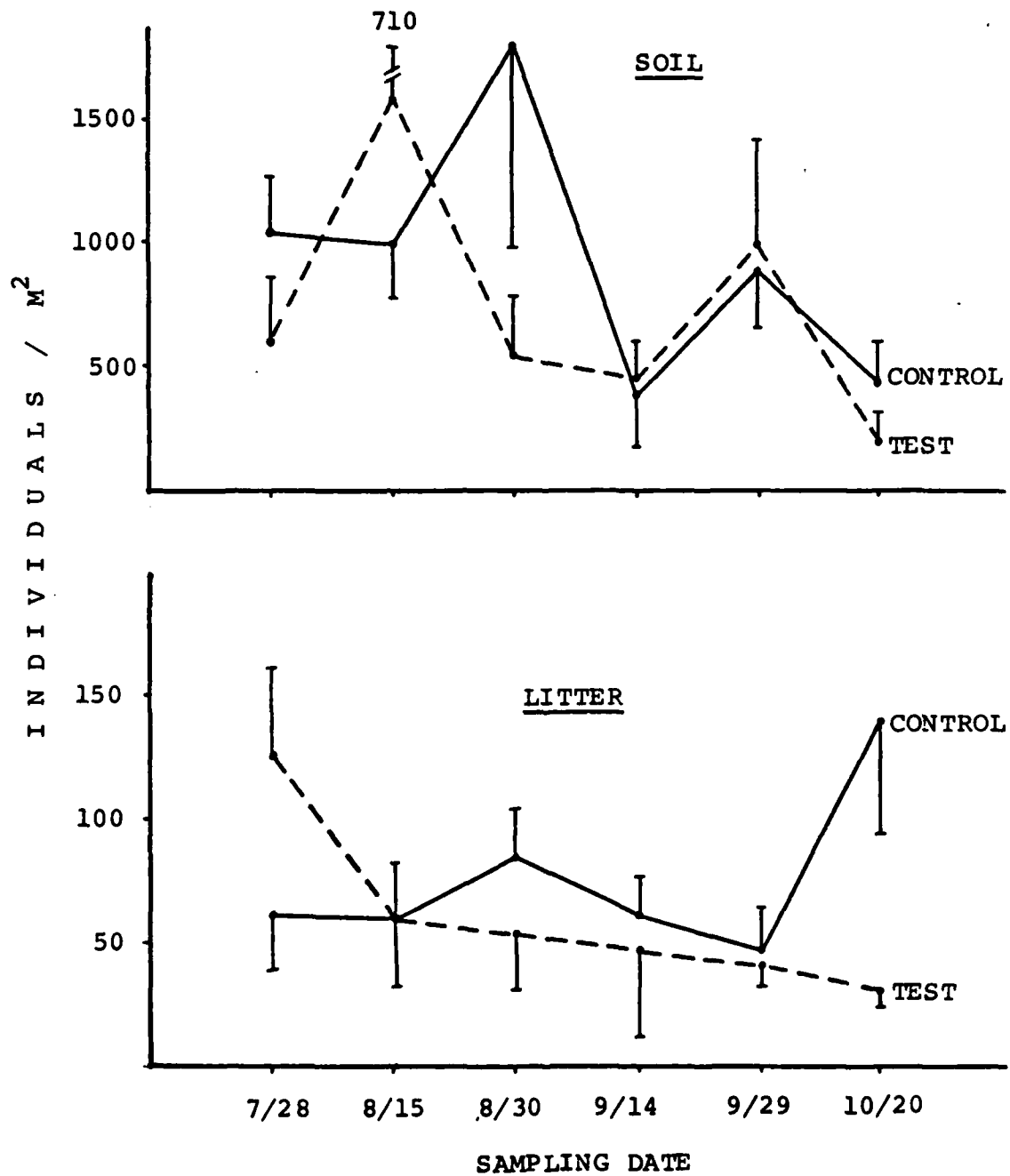


Fig. 8. Densities \pm SE of larval Diptera, 1983.

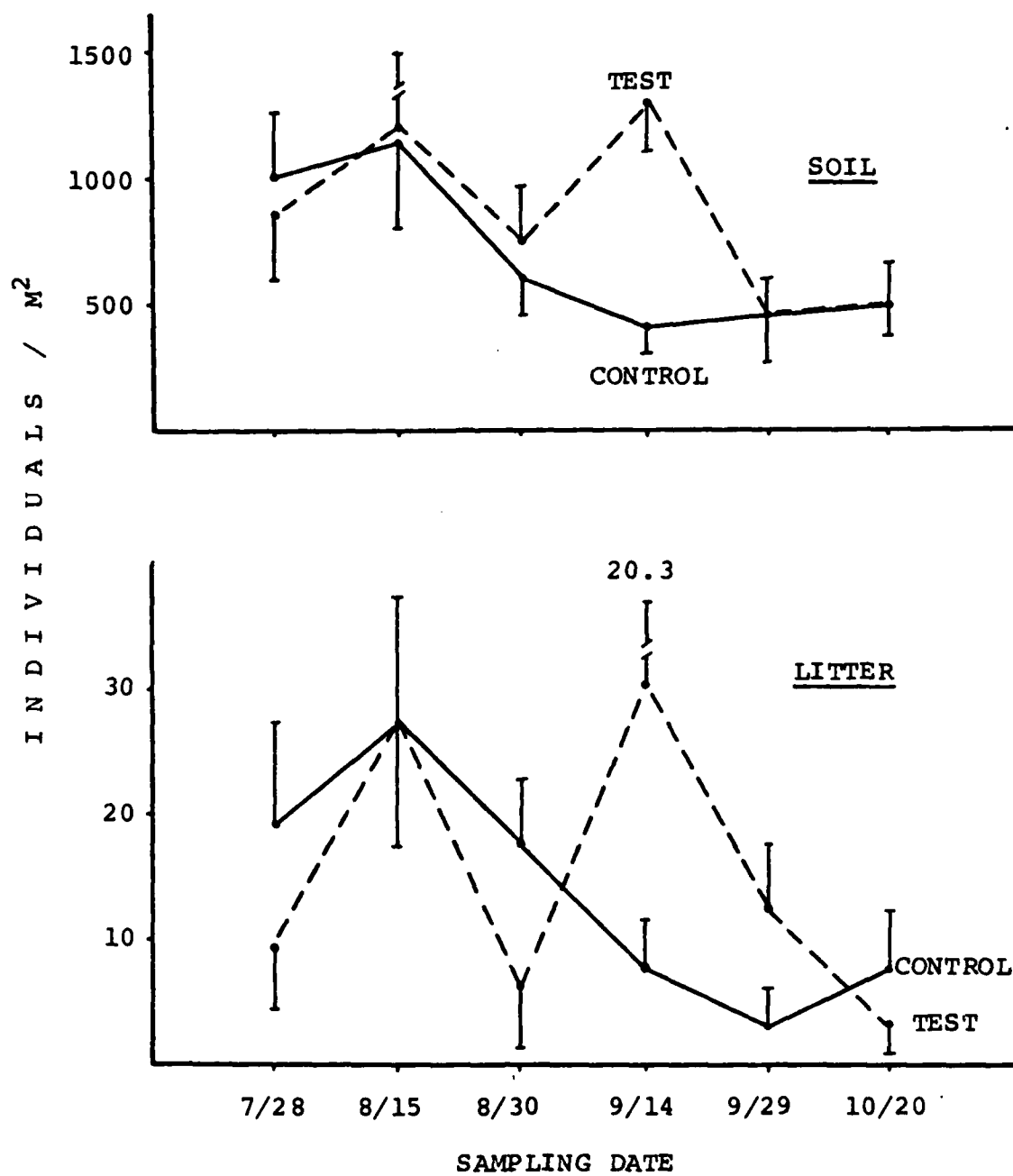


Fig. 9. Densities \pm SE of larval Coleoptera, 1983.

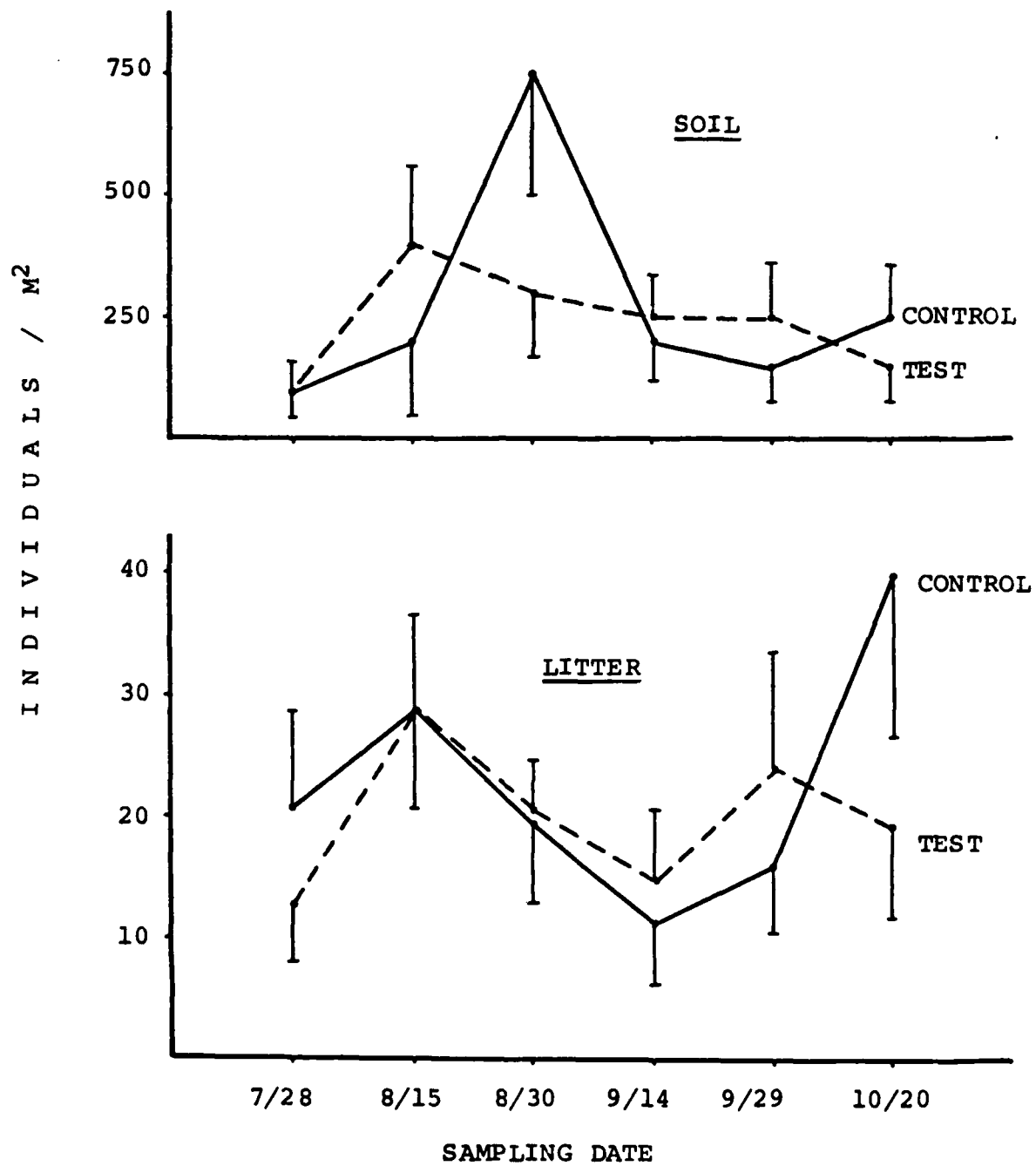


Fig. 10. Densities \pm SE of Aranei, 1983, in soil and litter.

from soil and litter were small Micryphantidae and Linyphiidae, and virtually all were immature. Pseudoscorpions, relatively abundant in both sites (Fig. 11), were restricted to leaf litter. Chilopoda, more abundant in Control than in Test, consisted mainly of geophilomorphs which do range into leaf litter, but preferentially live in the upper A layer.

At the order level, seasonal density fluctuations were generally not synchronous in the two sites, with some exceptions (e.g., soil-dwelling Collembola, Fig. 7). Identification of factors contributing to these site-specific density changes requires further taxonomic breakdown of abundant groups. As examples, selected taxa are further discussed below.

ii. Collembola:

Soil-inhabiting springtails were dominated by Onychiuridae, but Isotomidae and Entomobryidae also reached densities of several thousand / m² (Fig. 13). Hypogastruridae and Sminthuridae were less abundant, and Neelidae were not represented at all in September Control samples (Fig. 14).

Entomobryids, hypogastrurids and litter-dwelling sminthurids exhibited relatively synchronous density changes in the two sites. At the family level, preferences for soil or litter habitats were most pronounced in onychiurids, isotomids (Fig. 13) and hypogastrurids (Fig. 14). An apparent preference for soil in entomobryids was mainly due to one species, as will be shown below.

Data for two families, Entomobryidae and Sminthuridae, exemplify site comparison at the species level, which will not be completed until 1984 data are available.

Both sites harbored essentially the same association of species (Table 13), several of which showed similar dominance values within their family. S. henshawi clearly dominated among sminthurids in both sites.

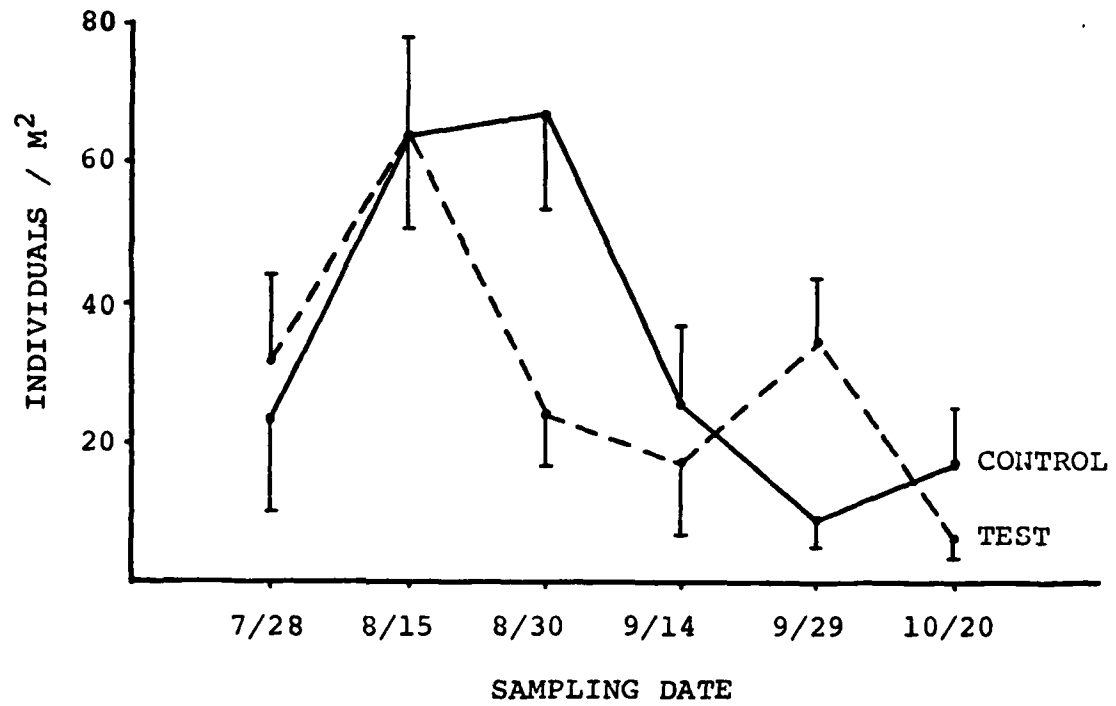


Fig. 11. Density \pm SE of Pseudoscorpiones, litter, 1983.

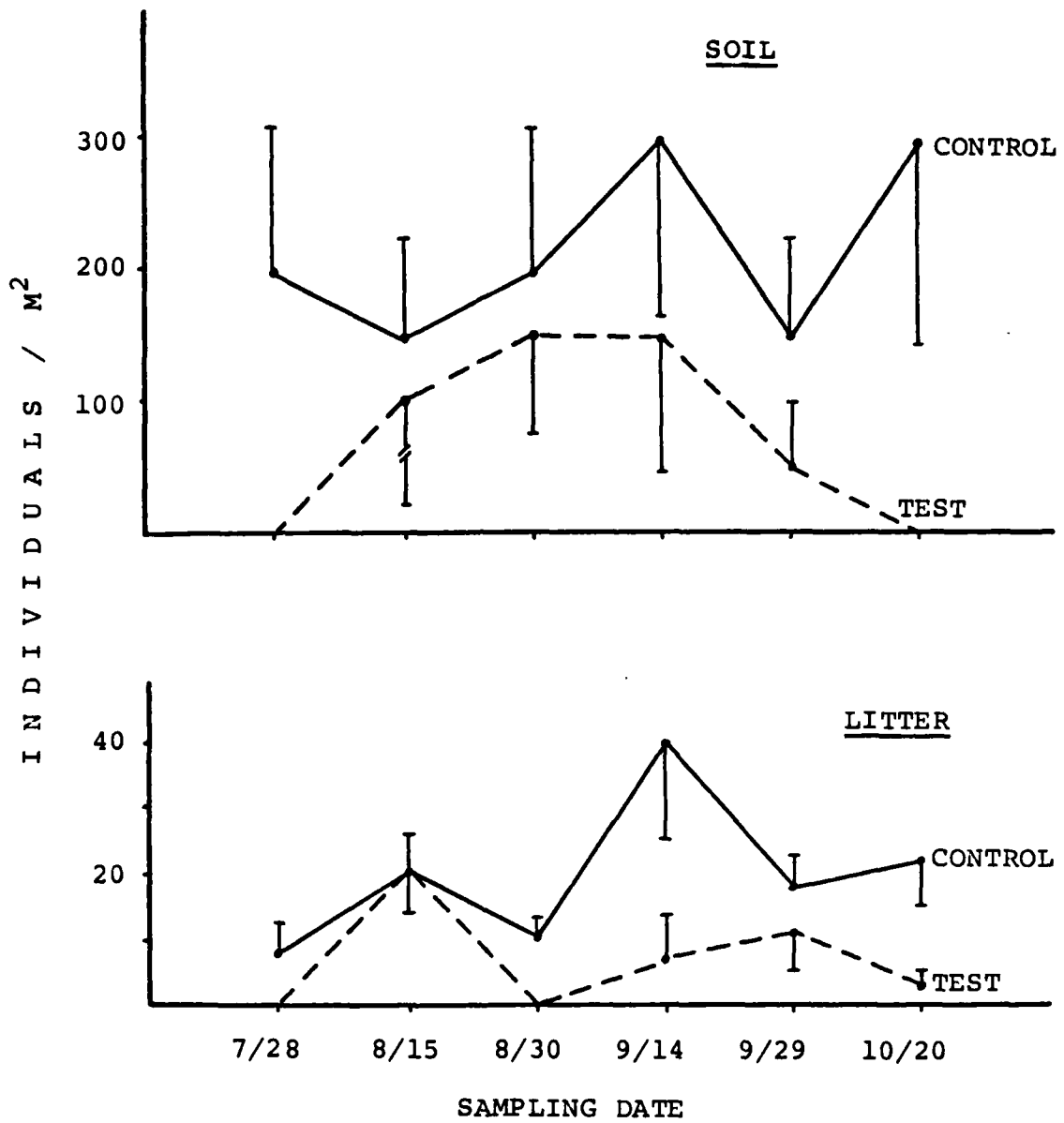


Fig. 12. Density \pm SE of Chilopoda in litter and soil, 1983.

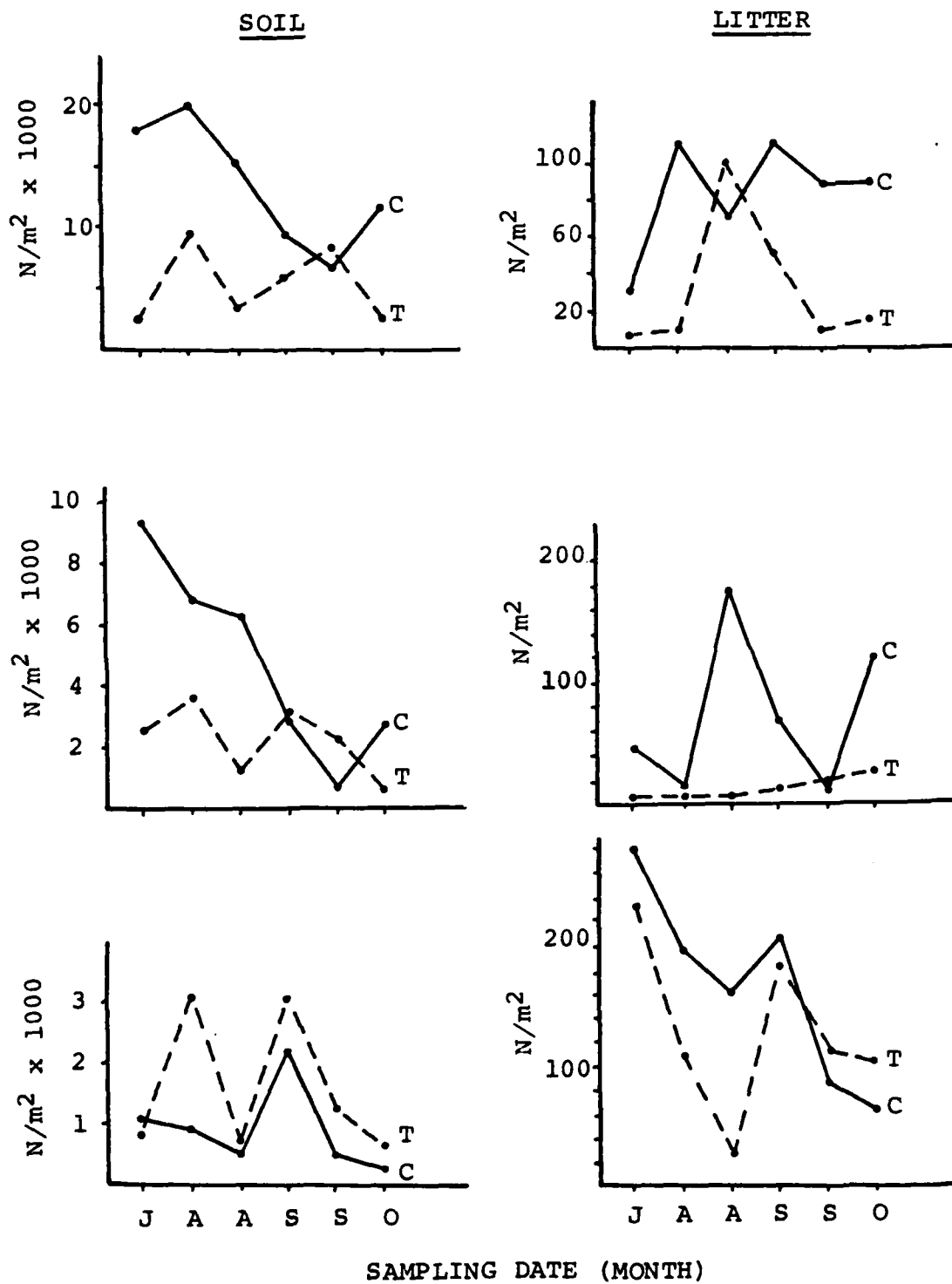


Fig. 13. Densities (SE omitted) of three most abundant families of Collembola in litter and soil, 1983 (T= Test, C= Control).

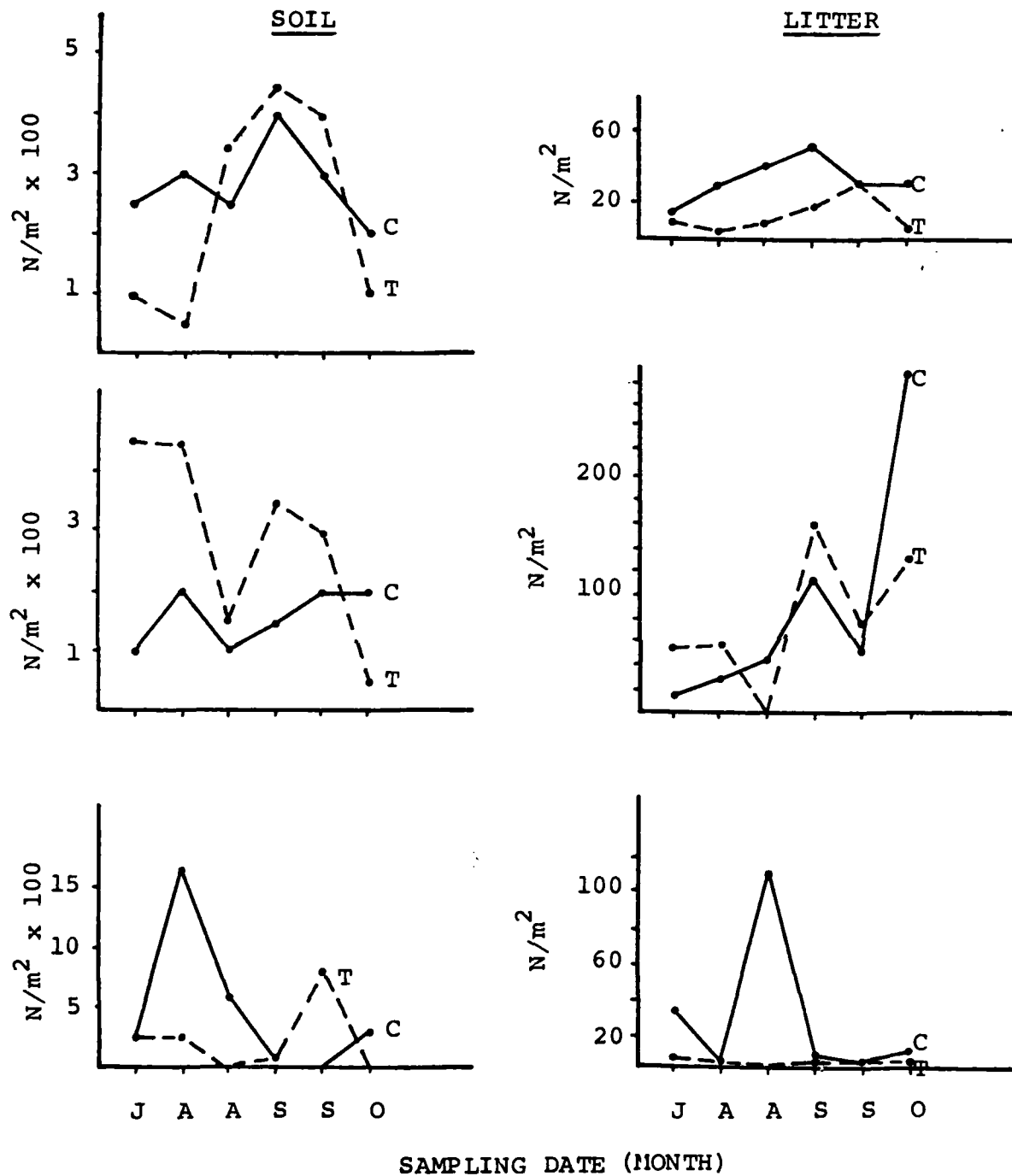


Fig. 14. Densities (SE omitted) of moderately abundant families Collembola in Test (T) and Control (C), litter and soil 1983.

Among entomobryids, T. flavescens was prevalent in Control, E. comparata (followed by T. flavescens) in Test. Three or four moderately common species are also shared between sites (Table 13).

Table 13. Dominance indices (% of total in each family) for sminthurid and entomobryid Collembola in Test and Control, summer-fall 1983.

	D	%
	Test	Control
Sminthuridae		
<u>Sminthurinus henshawi</u> (Folsom)	77.5	90.4
<u>Sminthurides lepus</u> Mills	2.7	0.5
<u>Arrhopalites benitus</u> (Folsom)	16.0	1.4
<u>A. caecus</u> (Tullberg)	0.3	1.0
<u>A. amarus</u> Christiansen	3.6	3.3
<u>Dicyrtoma aurata</u> (Mills)	-	3.3
Entomobryidae		
<u>Tomocerus flavescens</u> Tullberg	39.2	20.3
<u>T. lamelliferus</u> Mills	14.9	9.4
<u>Lepidocyrtus paradoxus</u> Uzel	12.4	17.5
<u>L. helenae</u> Snider	0.7	7.2
<u>L. lignorum</u> (Fabricius)	0.1	-
<u>Orchesella hexfasciata</u> Harvey	12.8	5.0
<u>Pseudosinella violenta</u> (Folsom)	9.1	-
<u>P. rolfsi</u> Mills	1.8	-
<u>Willowsia buski</u> (Lubbock)	0.7	0.2
<u>Entomobrya comparata</u> Folsom	3.3	36.2
<u>E. nivalis</u> (Linne)	3.0	3.5
<u>E. purpurascens</u> (Packard)	0.8	0.6

S. henshawi showed preference for leaf litter: the proportion of individuals in soil:litter was approximately 1:20 in both sites. Seasonal densities, appreciably different in Test and Control, increased in late fall (Fig. 15). Among litter-dwelling entomobryids, T. flavescens exhibited a late-summer density peak clearly mirrored in T. lamelliferus, but only in the Test site; Control populations were more stable (Fig. 16). E. comparata, sparsely represented in Test, exhibited two density peaks not comparable to those of other species (Fig. 17). Only populations of O. hexfasciata (Fig. 17), with a late-summer increase similar to Tomocerus spp., fluctuated synchronously in both sites. All litter-dwelling

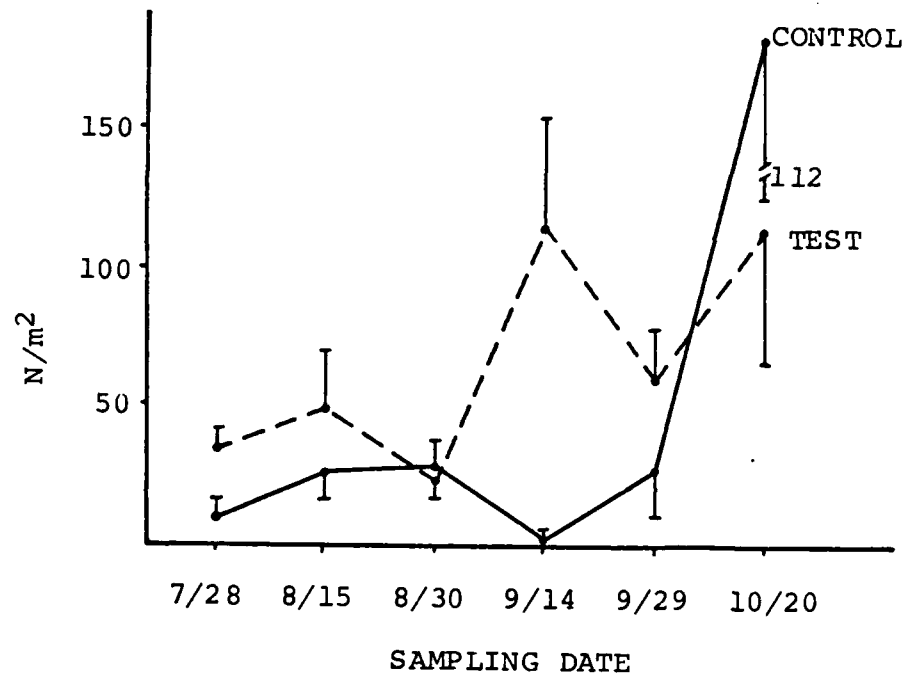


Fig. 15. Density \pm SE of *Sminthurinus henshawi* in leaf litter, 1983.

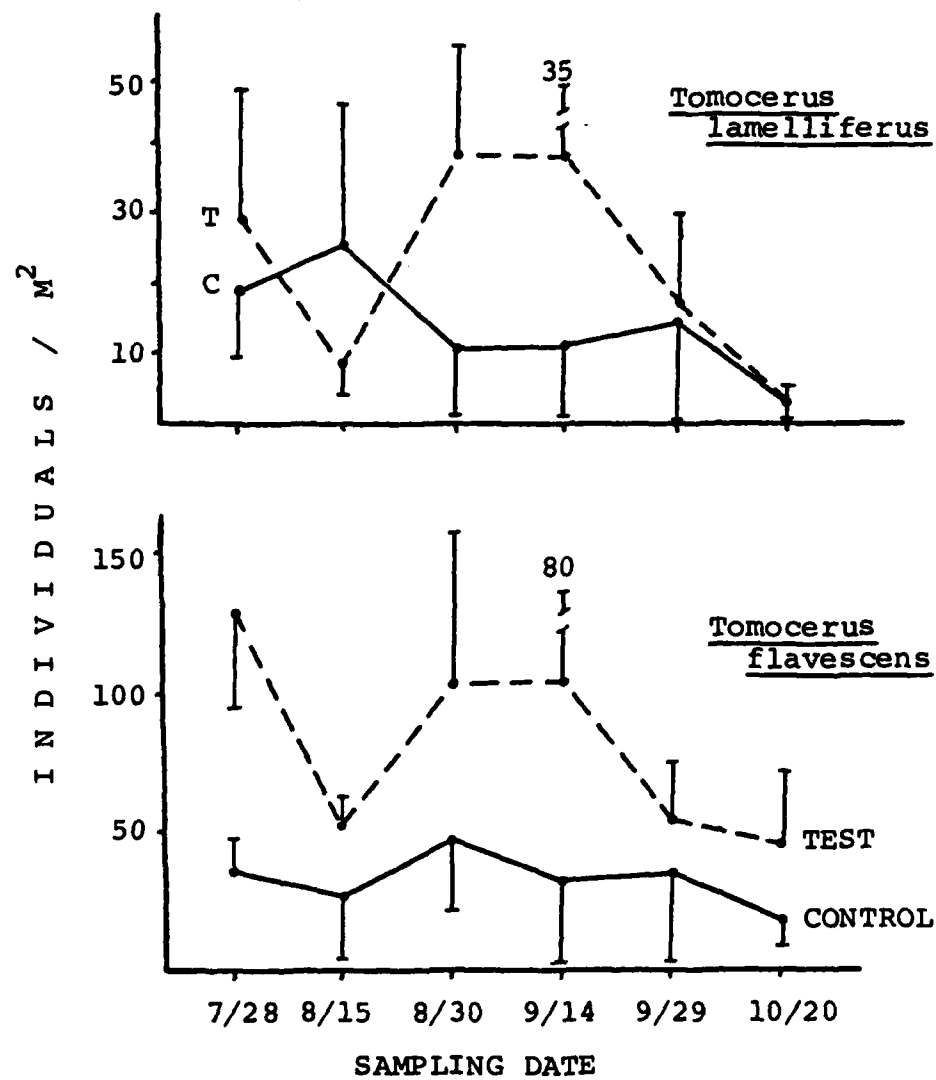


Fig. 16. Density \pm SE of two Tomocerus spp. in leaf litter, 1983.

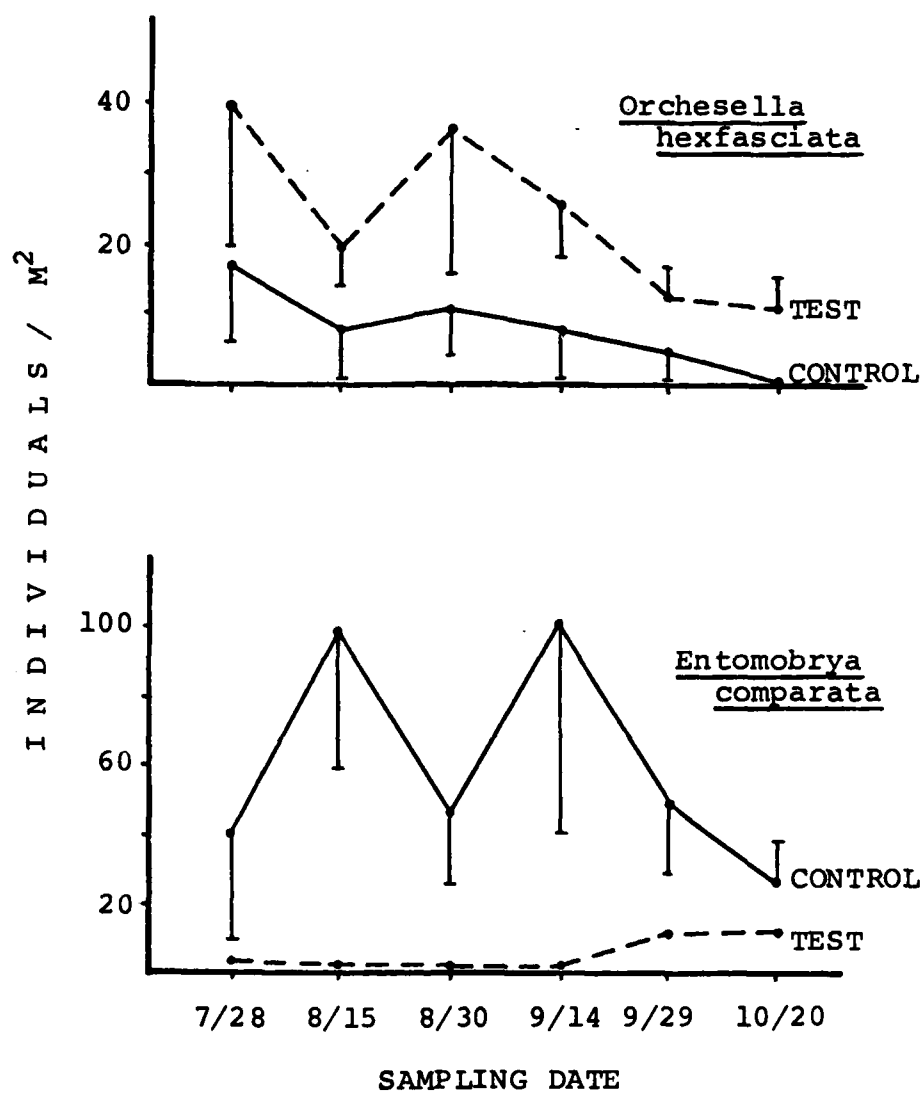


Fig. 17. Density \pm SE of two common entomobryids in leaf litter, 1983.

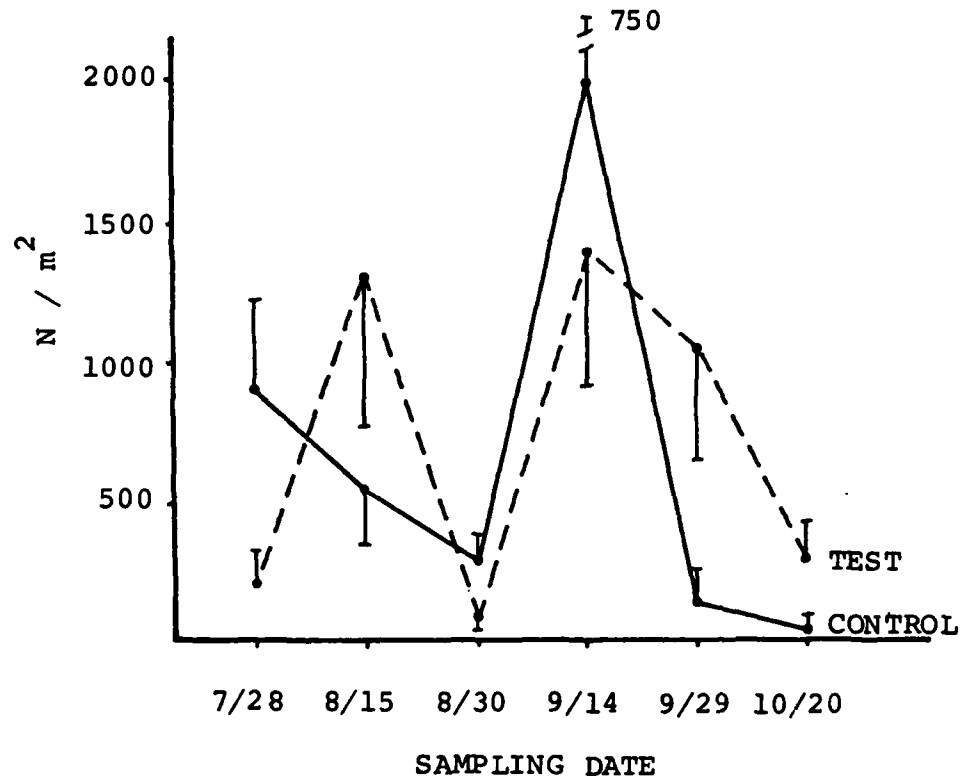


Fig. 18. Density \pm SE of Lepidocyrtus paradoxus in soil, 1983.

entomobryids were highly aggregated (standard errors frequently close to the means).

Of the species listed in Table 13, only two entomobryids were distinctly soil-dwelling: P. violenta, found so far only in Test, and L. paradoxus, abundant in both sites. The latter was primarily responsible for high entomobryid densities in soil: seasonal density fluctuations for the family (Fig. 13) in Test and Control were mainly due to those of L. paradoxus (Fig. 18).

iii. Acari:

1983 samples furnish a first assessment of acarine diversity and abundance in definitive sites. Taxonomic difficulties due to lack of pertinent keys are being overcome; at this time, mites are identified to family (not oribatids), Endeostigmata to genus, and distinctive or frequently occurring genera in other groups are also recorded.

As the reference collection becomes more complete and detailed, and numerical relationships between different taxa emerge, we will identify and focus effort on those groups which best serve project objectives. Below, soil-dwelling Acari are discussed with these considerations in mind.

In late-season 1983, total mite densities were generally greater in Control than in Test (Fig. 6), significantly so on August 30 and October 20 ($P \leq 0.02$). Density changes were synchronous until late September-October.

Oribatids underwent similar changes in Test and Control (Fig. 19). Prostigmata, dominating with peak densities of $>20,000/m^2$ (Table 14) differed widely between sites (Fig. 20). Among prostigmatid families, Rhagidiidae, Eupodidae and Scutacaridae were prevalent (Table 14). Scutacarids were distinctly clumped, occurring infrequently but in high numbers. Eupodids and rhagidiids were both more abundant in Control than in Test, but occurred with higher frequency than scutacarids in both sites.

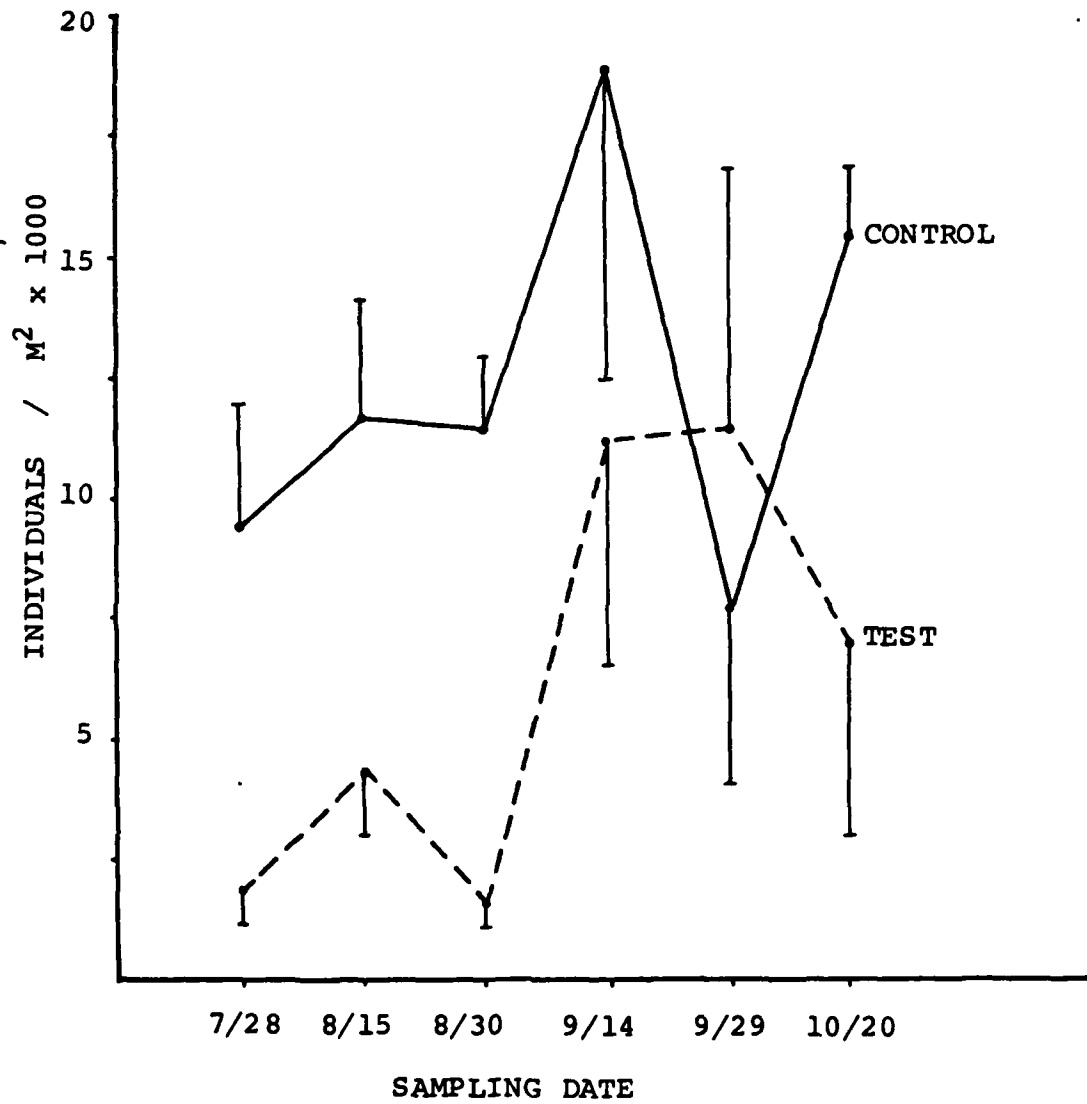


Fig. 19. Density \pm SE of Oribatida in soil, 1983.

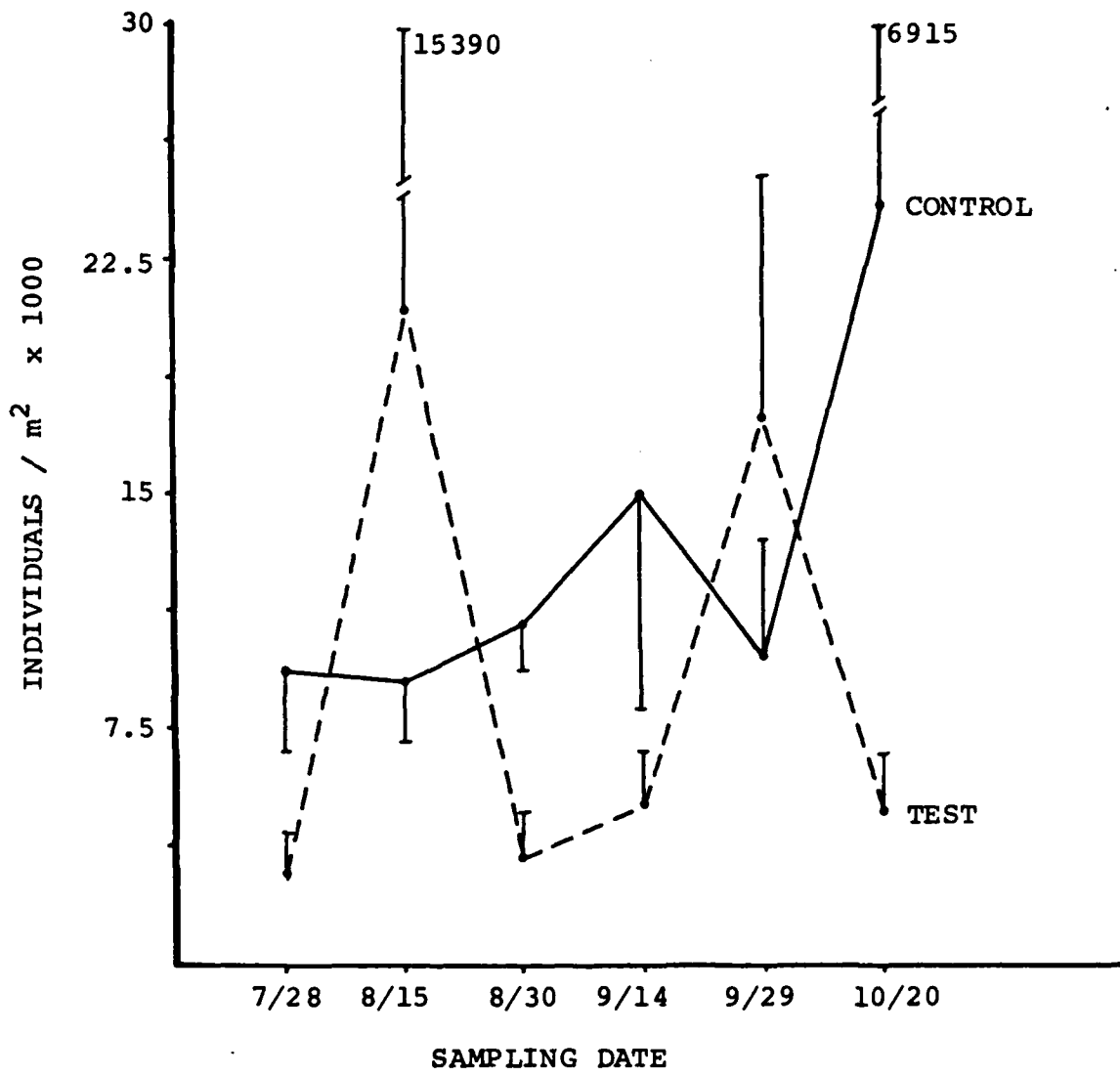


Fig. 20. Density \pm SE of Prostigmata in soil, 1983.

Pygmephoridae, prominent in Test only, also showed pronounced aggregation: found in 23% of samples overall, their dominance value of 34% (Table 14) was due to high numbers in only 5% of the samples. Indeed, the August 15 density peak in Test (Fig. 20) was due almost entirely to Pygmephoridae.

Mesostigmata, with diametrically opposed density fluctuations in Test and Control (Fig. 21), were dominated by one distinct genus which seems to consist of one or a few species ("species complex A", Table 14). Veigaia and Asca spp. were also shared between sites. Species complex A, showing dissimilar seasonal trends in Test and Control (Fig. 22), needs to be taxonomically separated before the data can become meaningful. The same is true of the most abundant endeostigmatid, Nanorchestes spp., in which density fluctuations in Test were a mirror image of those in Control (Fig. 23).

The bulk of Astigmata, relatively dense but also highly aggregated (Table 14), frequently stemmed from a single sample per date. In Test, 1% of all individuals were phoretic hypopi, versus 91% in Control. We do not know what the significance of this may be, since it is not known what triggers the development of hypopi (an optional developmental stage).

Year-to-year and between-site analyses based on higher taxa, as discussed above, would likely yield meaningless results. ANOVA of Prostigmata, for instance, or even of the small species group A, may show significant site/season interactions: i.e., simply confirm inverse seasonal trends in Test and Control (Figs. 20 and 22). It seems that site effects, and eventually ELF effects, should be described and analyzed in terms of genera and species. They need not be the same in Test and Control, but should belong to higher taxa abundant in both sites, and to the same general trophic group.

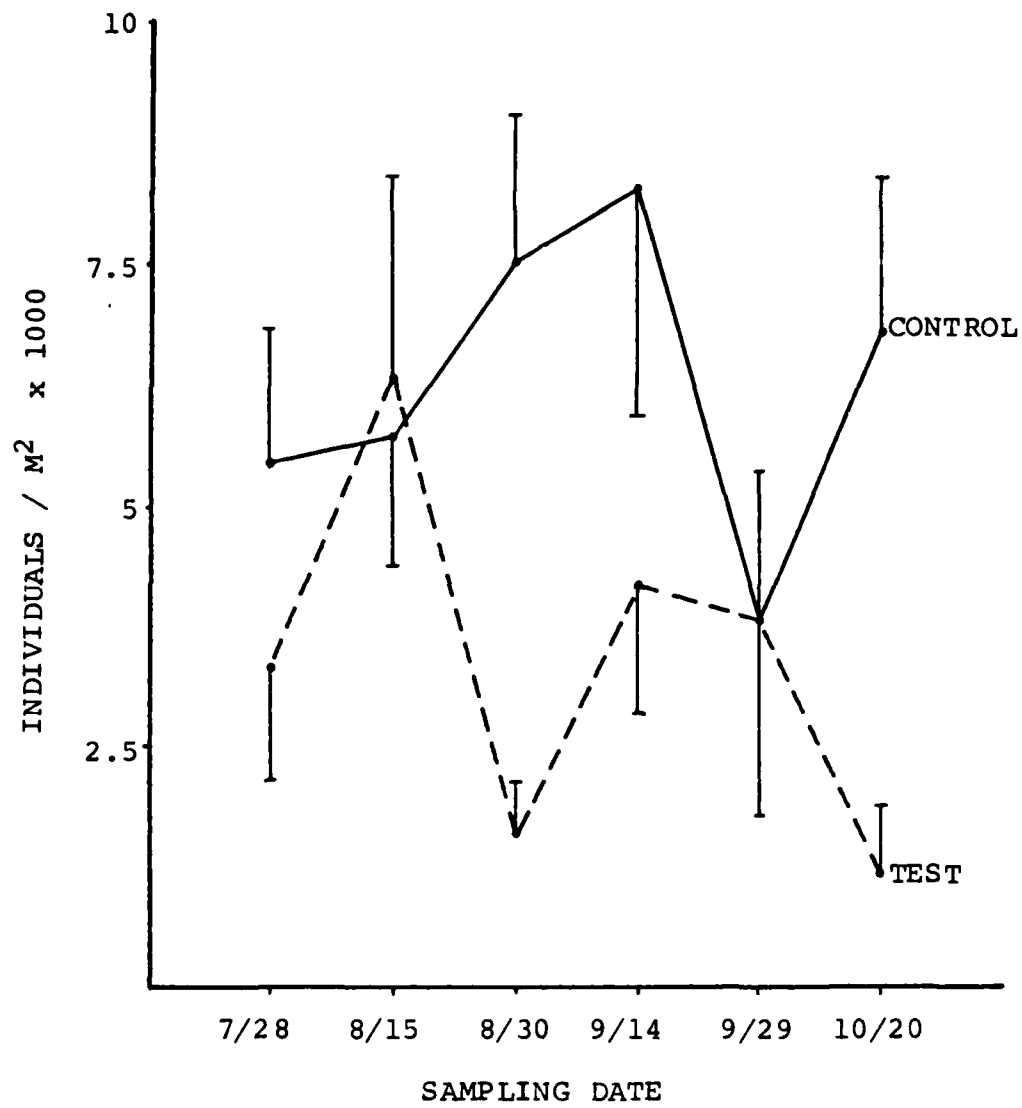


Fig. 21. Density \pm SE of Mesostigmata in soil, 1983.

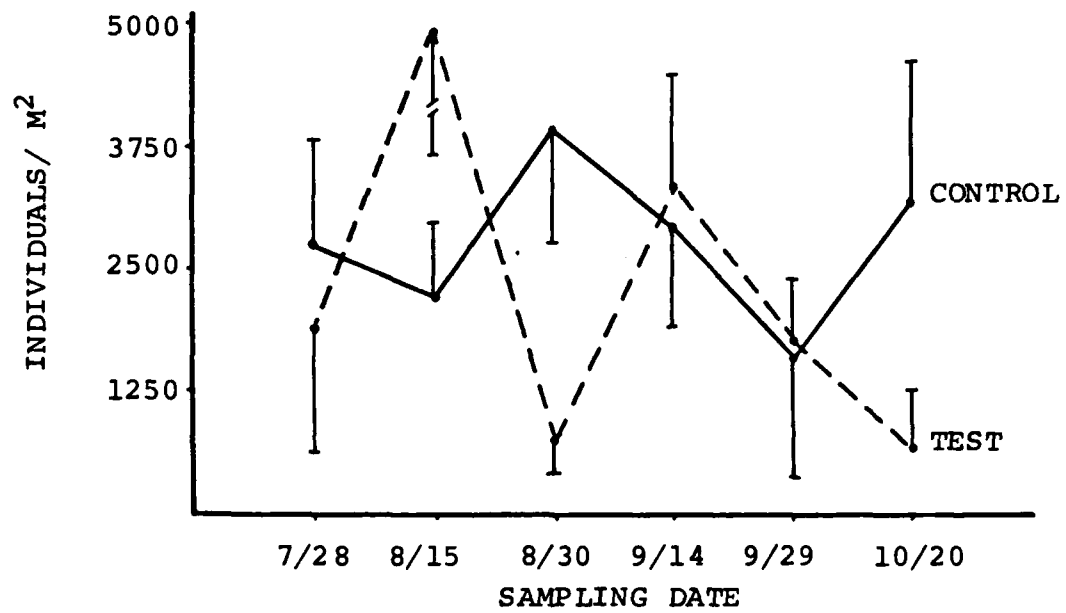


Fig. 22. Density \pm SE of "species complex A" (Mesostigmata) in soil, 1983.

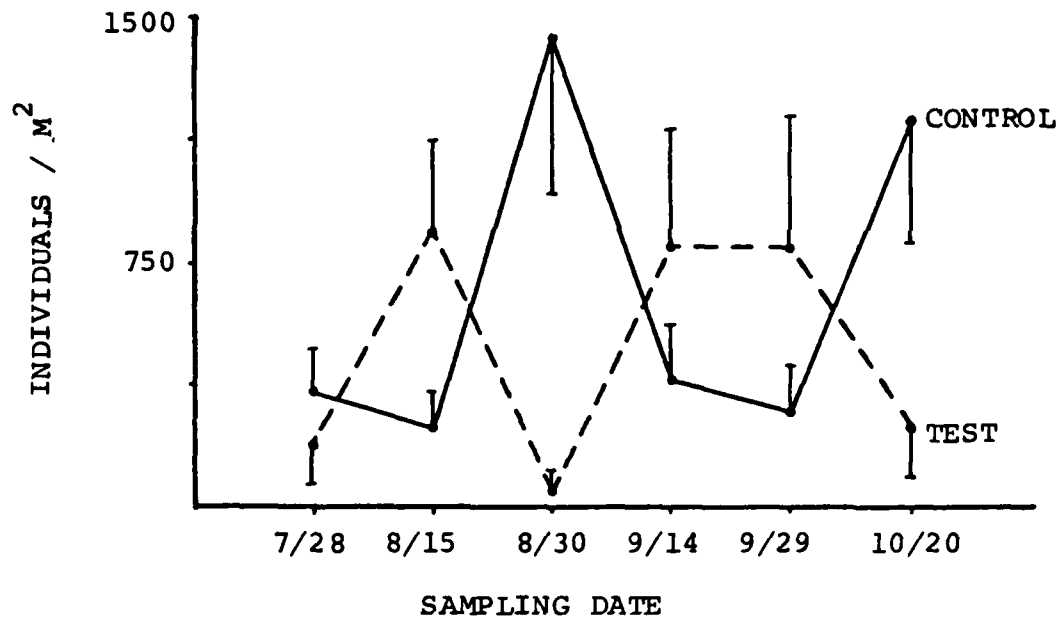


Fig. 23. Density \pm SE of *Nanorchestes* spp. in soil, 1983.

We have tentatively selected a few taxa for more intensive study, while still obtaining total numbers for all suborders:

a) Eupodidae and Rhagidiidae: numerically dominant Prostigmata in Test and Control (ignoring the highly variable Pygmephoridae in Test); analysis planned at the generic level, and at species level within dominant genera if relevant to Test/Control comparison.

b) Species complex A: their obvious prevalence among Mesostigmata (Table 14) makes it mandatory to further analyze this group. Laboratory culture may have to be attempted to supply immature specimens, so that population dynamics in the field can be described.

c) Asca spp.: selected as a second representative of Mesostigmata, of lesser abundance than spp. A, but relatively constant frequency. Analysis is planned at the species level.

d) Tentatively, pending 1984 data, we also include Trombidiidae and Erythraeidae: their activity patterns are very distinct, and their life cycle differs from all above. In three of the four genera so far identified, the larval stage is ectoparasitic on insects and arachnids, while nymphs and adults are predaceous.

One other criterion for selecting the taxa named above is that they are also extracted from leaf litter in appreciable numbers, and most are surface-active (e.g., Nanorchestes spp., Trombidiidae, Erythraeidae). We thus have the potential, through specialization, of documenting spatial and seasonal fluctuations in enough detail to make analyses valid.

Table 14. Peak densities, and month of its occurrence, of Acari in Control and Test soil, summer-fall 1983. D % indicates dominance relative to other taxa within the same suborder; + indicates low numbers; - denotes absence from samples.

	D %, month, peak density/m ² ±SE	
	T E S T	C O N T R O L
Oribatida	Sep, 11300±5290	Sep, 18800±6445
Prostigmata	Aug, 20700±15590	Oct, 24000±6915
Endeostigmata		
<u>Oehserchestes</u> spp	-	0.26 +
<u>Nanorchestes</u> spp	5.51, Aug, 850±270	5.05, Aug, 1450±470
<u>Alicorhagia</u> spp	1.31, Aug, 250±170	9.34, Oct, 2650±855
<u>Stigmalychus</u> spp	-	0.19 +
<u>Pachygnathus</u> spp	-	0.06 +
<u>Bimichaelia</u> spp	-	0.38 +
<u>Alycus</u> spp	-	0.19 +
<u>Hybolicus</u> spp	-	2.17 +
Eupodina		
Rhagidiidae	10.19, Sep, 1700±640	19.76, Oct, 3850±995
Tydeidae	6.07, Sep, 1600±760	4.60, Sep, 900±425
Eupodidae	26.36, Sep, 3850±1955	37.85, Sep, 7000±3280
Bdellidae	0.65, Sep, 250±110	1.21, Sep, 350±130
<u>Ereynetes</u> spp	0.19 +	0.26 +
Heterostigmata		
Pygmephoridae	33.64, Aug, 15500±15384	1.15, Aug, 350±250
Scutacaridae	14.77, Sep, 6950±6455	16.50, Oct, 7150±7150
Pyemotidae	0.28 +	0.32 +
Tarsonemidae	0.19 +	0.13 +
Eleutherengona		
<u>Stigmaeus</u> spp	-	0.51 +
<u>Agistemus</u> spp	-	0.06 +
<u>Eustigmaeus</u> spp	-	0.13 +
Parasitengona		
Erythraeidae	0.19 +	0.06 +
Trombidiidae	-	0.06 +
Trombiculidae	0.19 +	0.19 +
Mesostigmata		
Parasitidae	2.51 +	2.00 +
"Spp. A"	65.08, Aug, 4900±2050	44.21, Aug, 3900±1245
Ologamasidae	2.01 +	6.92 +
<u>Veigaia</u> spp	6.53, Jul, 550±275	10.92, Sep, 1250±550
Ascidae	1.26 +	1.33 +
<u>Antennoseius</u> spp	-	0.13 +
<u>Asca</u> spp.	12.06 +	9.59 +
Zerconidae	1.01 +	8.66 +
Uropodoidea	-	0.13 +
Polyaspidioidea	4.77 +	1.73 +
Macrochelidae	1.76 -	-
Astigmata	Jul, 4850±4295	Aug, 14600±14545

IV. DIEL AND SEASONAL ACTIVITY

1. METHODS

Schedule of trap activation, disposition of traps relative to each other, and trap design are the basic elements of any trapping program. The weekly schedule we have used to date proved satisfactory for documenting activity patterns of the common species. Trap disposition was tested before definitive sites were available, in a short-term study in 1982: although no longer pertinent to the experimental design we now use, results are appended as a manuscript (submitted to *The Great Lakes Entomologist*). Trap design was tested in 1984, both concerning the use of funnel inserts and the effects of barriers on catch size and diversity: preliminary data are given below.

i. Effects of funnel inserts:

Standard pit traps used to date are provided with a funnel insert with a bottom opening diameter of 2.5 cm. The size of the opening, possibly aided by the less abrupt slope provided by the funnel, may affect capture rates of large or long-legged species.

In August 1984, this possibility was tested in a short-term experiment: 20 traps (10 pairs, 10 m distance between pairs, 50 cm distance between members of each pair) were set out in the vicinity of the Control site. One member of each pair was a standard trap, the other a no-funnel trap. They were activated on five consecutive days.

A summary of catch totals showed that funnels had no effect on trap efficiency. Total arthropods and total catches of the common groups were essentially equal (Table 15). The larger number of Hemiptera in no-funnel traps was due to 21 individuals entering a single trap.

Funnels have to be manipulated and cleaned every time traps are activated and emptied. Since their presence seems to bring neither advantage nor disadvantage, we will no longer use them in the future.

Table 15. Arthropod catches in pit-traps with and without funnel insert (n=10 traps each); means based on total catch/trap over five days (August 20-24, 1984). Winged Diptera and Hymenoptera, and hypopi, excluded.

	FUNNEL	NO FUNNEL
Opiliones	20	6
Aranei	43	56
Acari	444	492
Collembola	677	624
Hemiptera	31	52
Coleoptera	98	92
N Arthropoda	1496	1485
mean/trap \pm SE	149.6 \pm 8.3	148.5 \pm 13.6

11. Effects of barriers:

Adjacent to Control, two grids were measured and marked in a pattern mimicking that of the site (11.5 m distance between any two traps). Twenty standard traps were installed in one grid; the other contained 20 traps provided with four 1 m barriers (plastic garden edging) set in a cross-fence design at right angles to each other. Barriers of all traps were aligned in the same direction (Fig. 24), with minor deviations due to local obstructions, and protruded approximately 6 cm aboveground. Diel collecting schedule was the same as that for within-site traps (dusk to dawn to dusk once a week).

Half of the catches (all even-numbered traps of each kind) were sorted; the remaining material was stored and is available if needed. The following taxa were selectively sorted out: Opiliones, Aranei, Carabidae, Collembola, and velvet mites (Erythraeidae and Trombididae). Phoretic hypopi were counted separately, all other specimens were lumped as Miscellaneous.

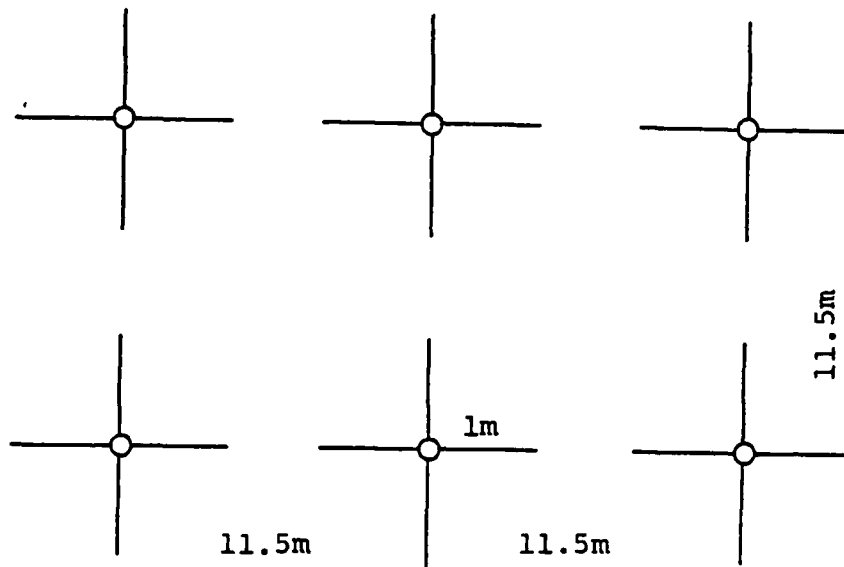


Fig. 24. Cross-fence design of barriers leading to pit-traps. Distances not to scale.

Results:

As expected, overall trapping efficiency was increased significantly by barriers: doubled in day traps, slightly more than doubled in night traps (Table 16). The magnitude of barrier effects varied with taxon, however, ranging from a >5x increase for carabids and velvet mites to <2x for Collembola, consistently so in both night and day traps (Table 16). Adult Opiliones, which we had hoped to catch at much higher rates by barriers, were apparently not abundant enough for accumulation of significant numbers.

Table 16. Total catches of selected arthropods in diel traps with and without barriers, each total derived from 240 traps (10 traps x 24 dates). B, barriers; NB, no barriers. Winged insects other than Coleoptera, and hypopi, excluded.

	NIGHT			DAY		
	B	NB	B/NB	B	NB	B/NB
Opiliones	98	48	2.04	13	6	2.16
Aranei	636	253	2.51	411	111	3.70
Velvet mites	128	24	5.33	328	61	5.38
Carabidae	1047	203	5.16	271	51	5.31
Collembola	2665	1553	1.71	3523	2025	1.74
Miscellaneous	2817	1360	2.07	3252	1680	1.94
TOTALS	7391	3441	2.15	7798	3934	1.98

Distribution of catches over time, in terms of absolute numbers captured of each major group, is shown in Figures 25-29. Barriers obviously enhance trapping efficiency, although their taxon-specific effects have to be taken into consideration when interpreting data. In order to take full advantage of the available material, breakdown to species level will be necessary. We thereby expect to increase the data base on species common to both Test and Control, so far caught in insignificant numbers.

In conclusion, we will use barrier trapping in the future, with the same

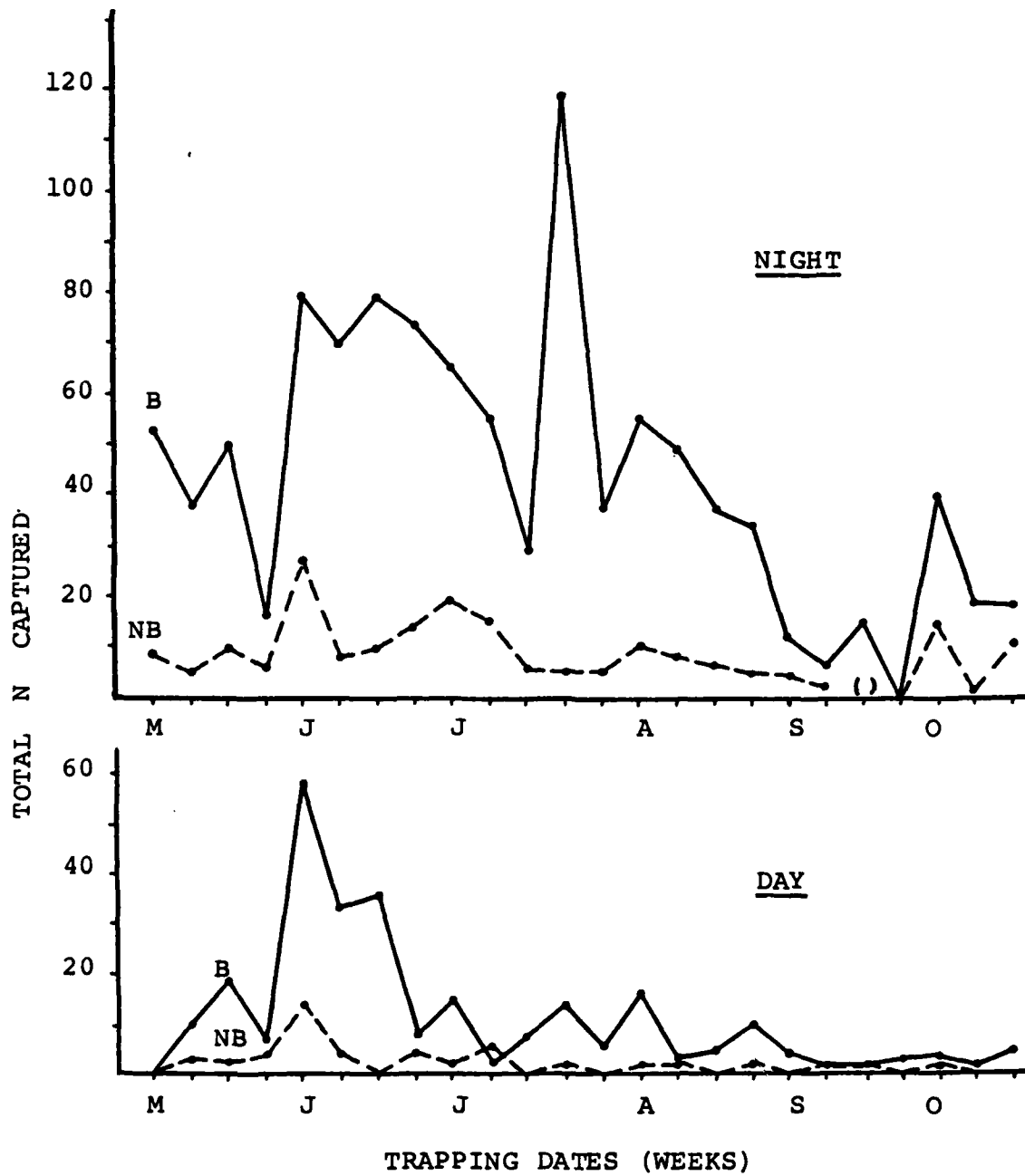


Fig. 25. Total diel catches of Carabidae in traps with barriers (B) and without them (NB).

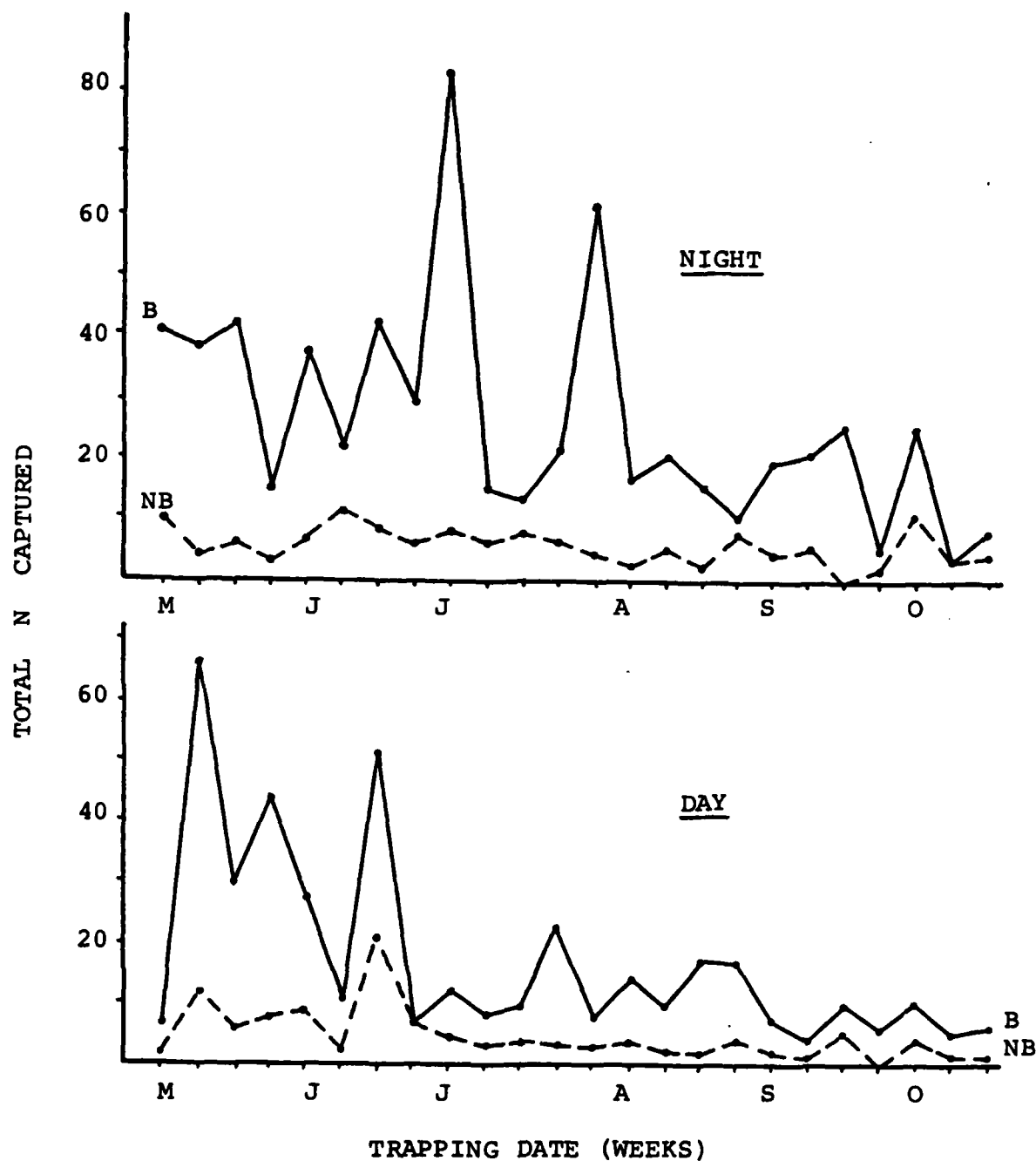


Fig. 26. Total diel catches of Aranei, 1984, in traps with barriers (B) and without barriers (NB).

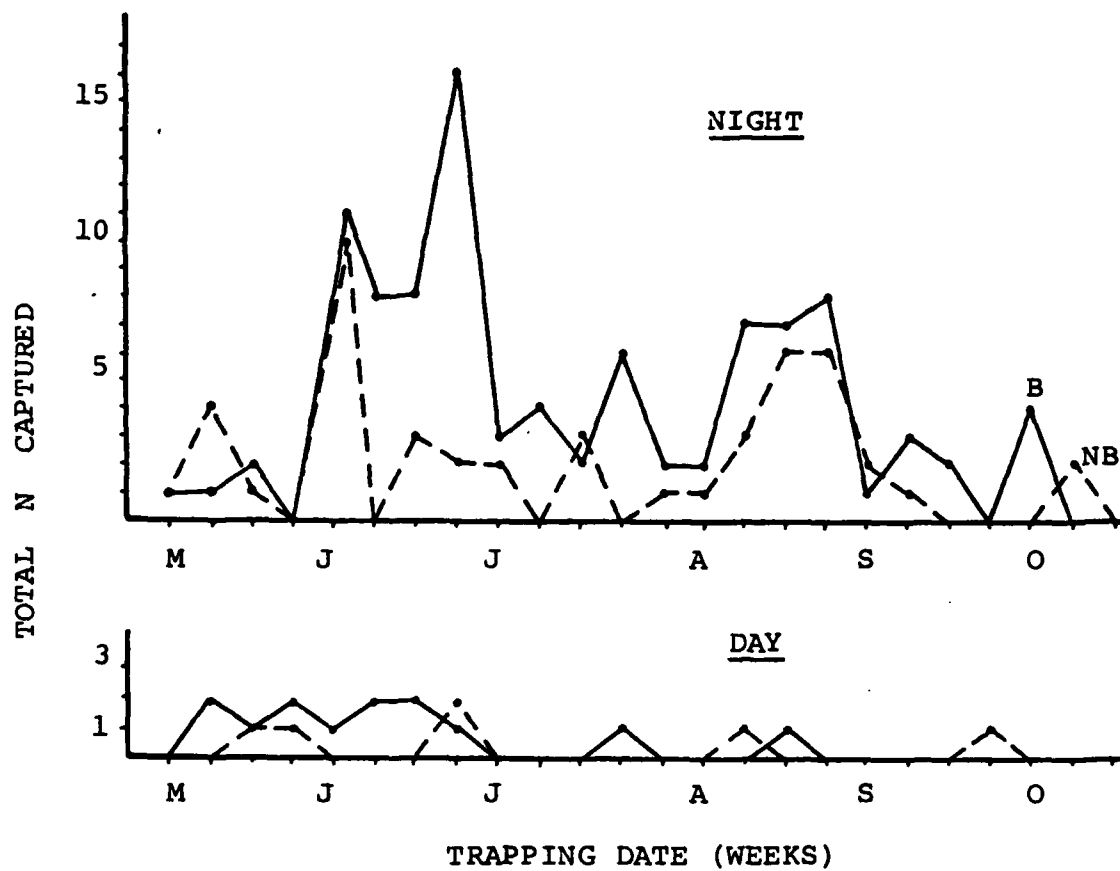


Fig. 27. Total diel catches of Opiliones, 1984, in barrier- (B) and non-barrier (NB) traps.

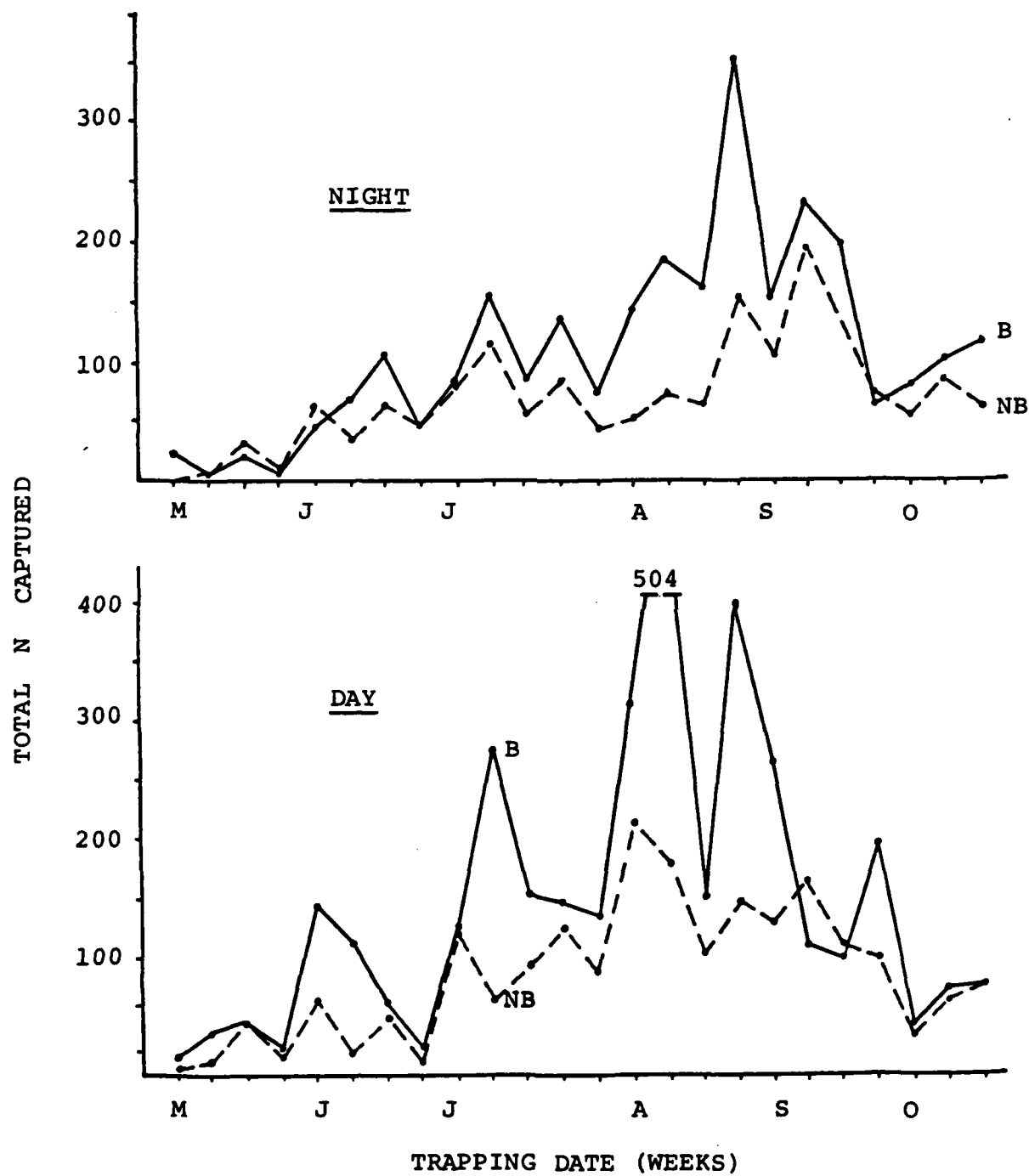


Fig. 28. Total diel catches of Collembola in barrier- (B) and non-barrier (NB) traps, 1984.

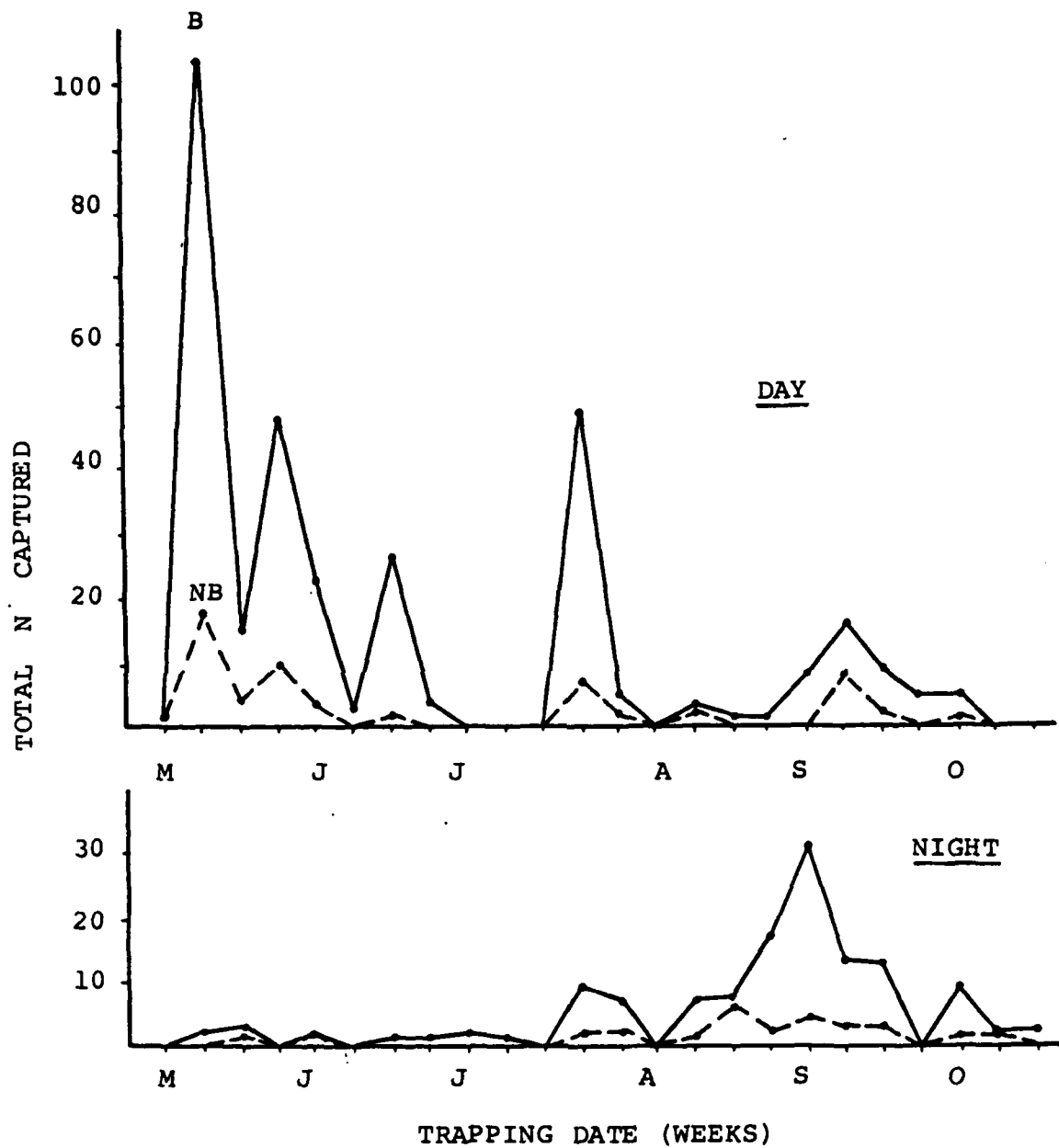


Fig. 29. Total diel catches of velvet mites (Trombidiidae and Erythraeidae) in barrier- (B) and non-barrier (NB) traps, 1984.

replication (20 samples/site/date). Identification of large catches is, however, labor-intensive. 1984 data, both from barrier and routine trapping, will therefore be used to determine the number of samples to be sorted and identified; cumulative (species/trap) and (individuals of a species/trap) curves will indicate the number necessary for: a) assessing diversity of the surface-active fauna, and b) obtaining enough individuals of less common species (dominance, say, 5-10%) for interpretation of activity patterns.

2. SURFACE-ACTIVE ARTHROPODS

1. Collembola:

We here discuss the families dealt with earlier, Sminthuridae and Entomobryidae, which comprise the dominant surface-active species.

In summer and fall of 1983, overall catch totals were similar in Test and Control (about 1600 individuals in each), but distribution over the two families differed. Both were almost equally represented in Control; in Test, entomobryids outnumbered sminthurids 3:1. Sminthurinus henshawi dominated surface-active sminthurids (approx. 80% in both sites), T. flavescens the entomobryids (around 50%), with O. hexfasciata in second place (Table 17).

Sminthurids were preferentially day-active, entomobryids nocturnal (Table 17). Different species exhibited different degrees of plasticity, however. During a cold spell in September, when daily minima were close to 0 C, S. henshawi became exclusively diurnal (Fig. 30). Both common entomobryids remained partly nocturnal, but generally decreased their activity (Figs. 31 and 32). In early October, activity increased briefly in the two entomobryids, and S. henshawi became partly nocturnal again; at the time, minimum temperatures had again risen to about 10 C (temperature

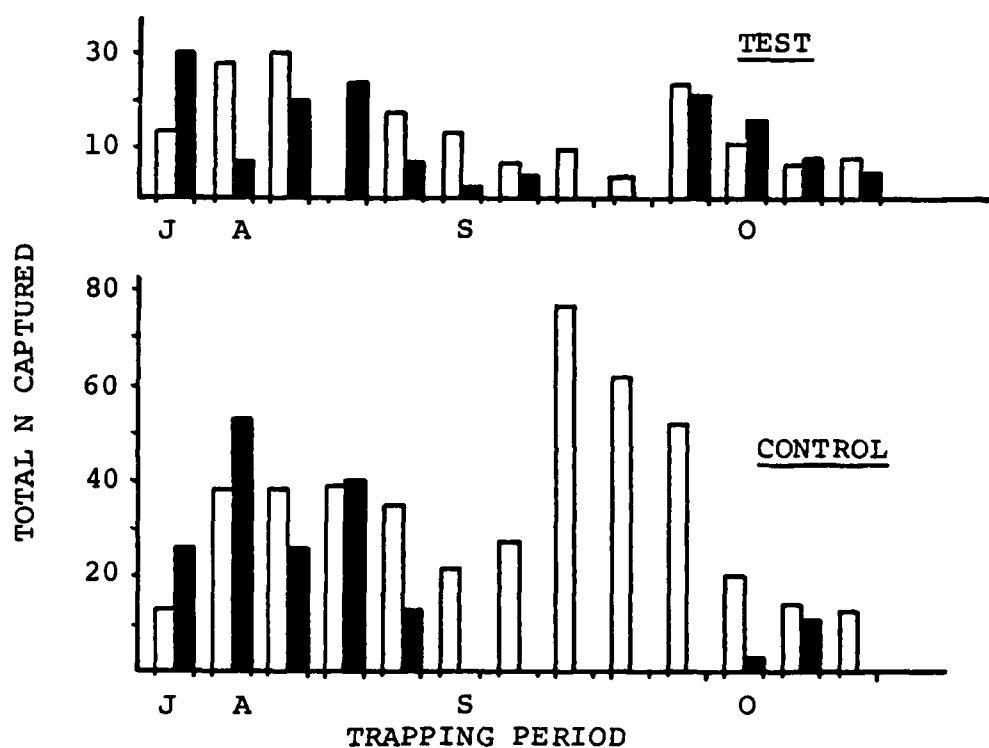


Fig. 30. Weekly diel catches of Sminthurinus henshawi in Test and Control, 1983. Open bars: day, black bars: night catches.

Key to dates:

July: 27-28;

August: 3-4; 10-11; 17-18; 24-25; 31-Sep 1;

September: 7-8; 13-14; 21-22; 28-29;

October: 5-6; 13-14; 26-27.

Test day samples, August 17, not taken because of vandalism.

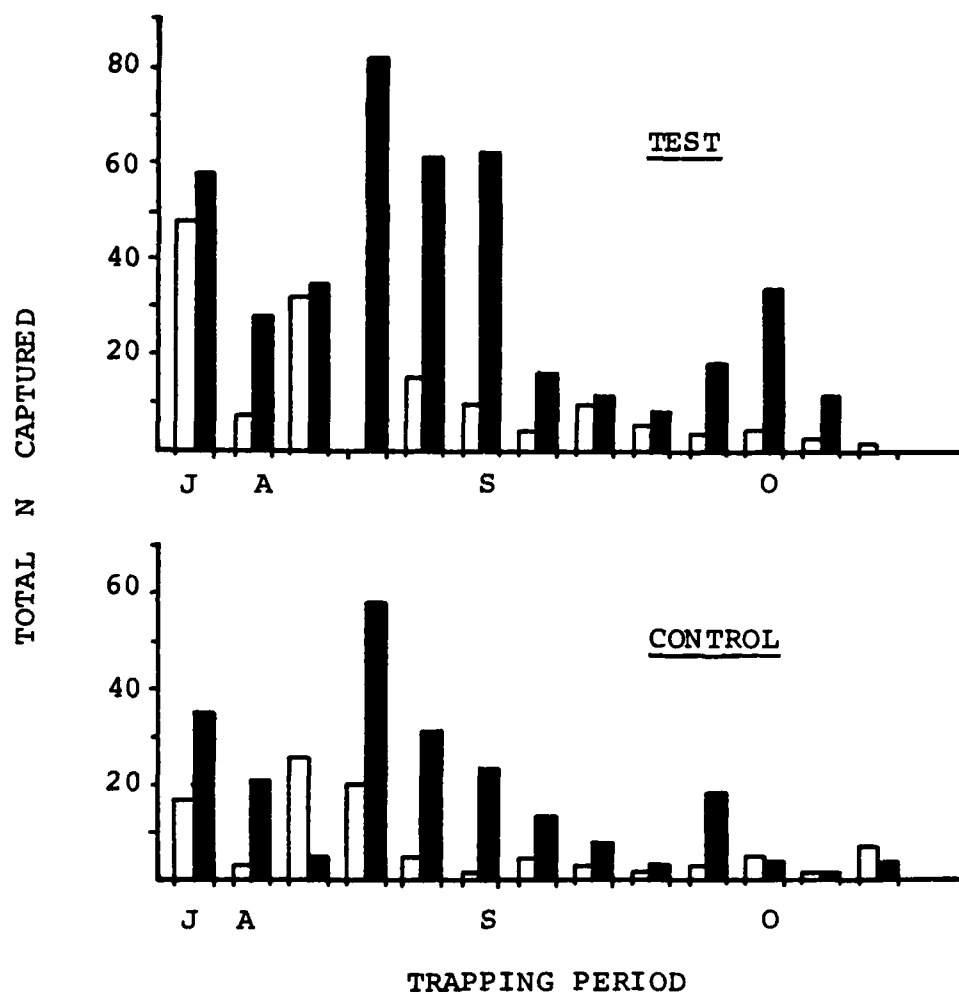


Fig. 31. Weekly diel catches (open bars: day; black bars: night) of Tomocerus flavescens in Test and Control, 1983. For exact dates, see Fig. 30.

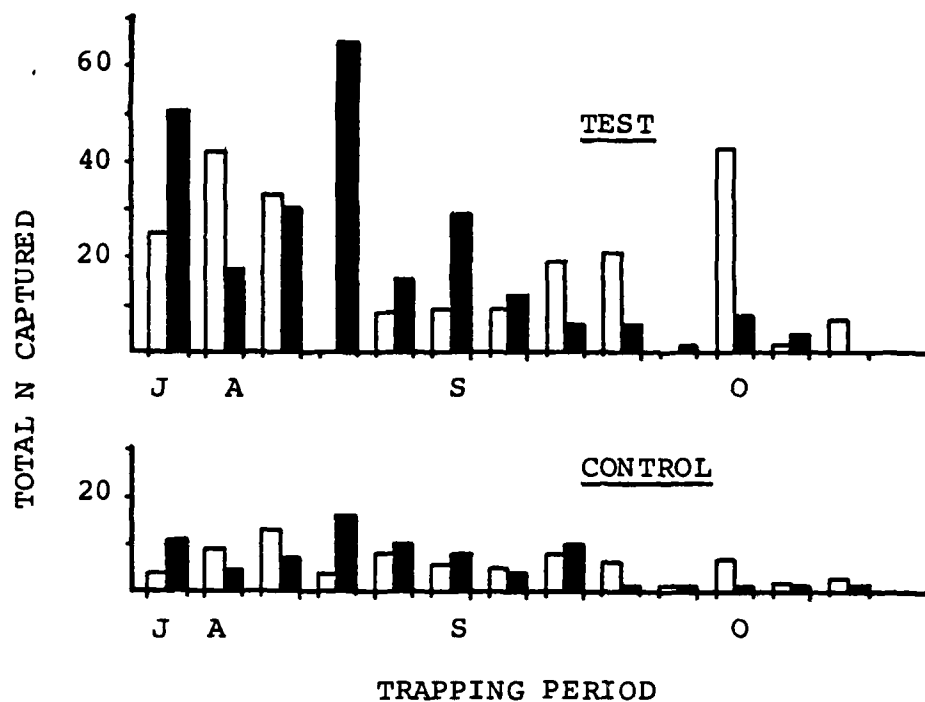


Fig. 32. Weekly diel catches (open bars: day; black bars: night) of Orchesella hexfasciata In Test and Control, 1983. For exact dates, see Fig. 30.

records from a nearby National Weather Station).

Other species, many shared between sites, were caught infrequently or in low numbers. Once 1984 material, and barrier-trapped specimens, have been identified, we will analyze climate-dependent behavior of more than just the dominant species. Barrier-trapping in Test and Control in the coming season will eventually expand analyses to between-site comparisons based on larger numbers of specimens.

Table 17. Total numbers of sminthurids and entomobryids trapped in 1983, relative occurrence of common species, and distribution of catches over night and day for each family.

	TEST	CONTROL
Sminthuridae		
total N	396	788
% nocturnal	41.9	32.4
% diurnal	58.1	67.6
% <u>S. henshawi</u>	80.8	78.8
Entomobryidae		
total N	1208	610
% nocturnal	63.6	61.1
% diurnal	36.4	38.9
% <u>T. flavescens</u>	46.4	52.8
% <u>O. hexfasciata</u>	38.1	24.9

ii. Carabidae:

In late summer and fall of 1983, the carabid fauna was more diverse in Control than in Test. Of 15 species total, ten were shared; five, all uncommon, were unique to Control (Table 18).

Total catches, i.e. total carabid active densities, were equal in the sites. Not so at the species level: Test carabids were heavily dominated by P. melanarius; in Control, five species were almost equally abundant (13.8-20.0 %), including P. melanarius (Table 18). Both species richness and evenness thus differed.

Table 18. Carabidae active in Test and Control, late season 1983, and % occurrence for each.

	TEST	CONTROL
TOTAL N INDIVIDUALS	487	484
<u>Pterostichus melanarius</u> Illiger	82.3	19.2
<u>P. coracinus</u> Newman	5.1	20.0
<u>P. pensylvanicus</u> Leconte	7.0	17.4
<u>Calathus ingratus</u> Dejean	1.0	13.8
<u>C. gregarius</u> Say	0.8	8.3
<u>Clivina fossor</u> Linne	0.8	0.2
<u>Synuchus impunctatus</u> Say	1.6	14.7
<u>Agonum retractum</u> Leconte	0.2	0.8
<u>A. decentis</u> Say	0.6	0.2
<u>Harpalus fuliginosus</u> Duftschmid	0.4	0.2
<u>P. adstrictus</u> Eschscholtz	-	3.1
<u>P. adoxus</u> Say	-	1.0
<u>Cymindis cribricollis</u> Dejean	-	0.4
<u>Myas cyanescens</u> Dejean	-	0.4
<u>Sphaeroderus lecontei</u> Dejean	-	0.2

The six most frequently captured species (in at least one site) all tended toward night-activity. Among them, C. ingratus and C. gregarius were strictly nocturnal (Fig. 33), the other four were somewhat diurnal as well (Figs. 34-36).

Once 1984 data are completed, carabid active densities in Test and Control will be fully described and analyzed by multiple regression of catches on climatic factors. 1984 barrier-trapped material will supplement information for moderately common species..

iii. Aranei:

Summer-fall 1983 provided a first comparison of spider faunas in Test and Control. Twenty-three species were collected in each site; 15 were common to both, while a group of 8 species was unique to each (Table 19). All lycosids, thomisids and agelenids were common to both sites. Overall, these equally diverse faunas thus shared about 65% of their specific members, and these shared species included all of the most abundant (Table 19). Full-year (1984) data will no doubt alter this first assessment.

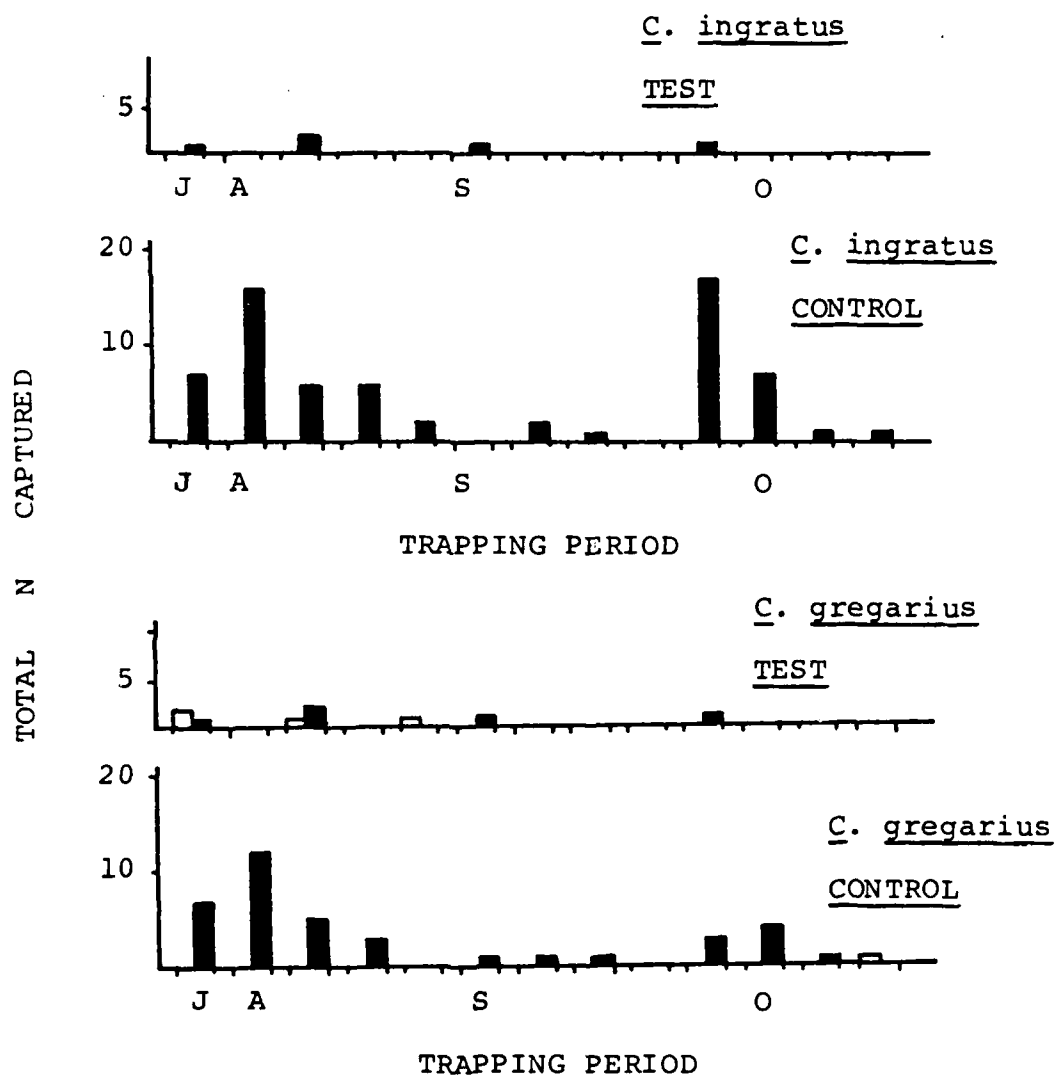


Fig. 33. Weekly diel activity of Calathus ingratus and Calathus gregarius, 1983 (open bars: day, black bars: night). For exact dates, see Fig. 30.

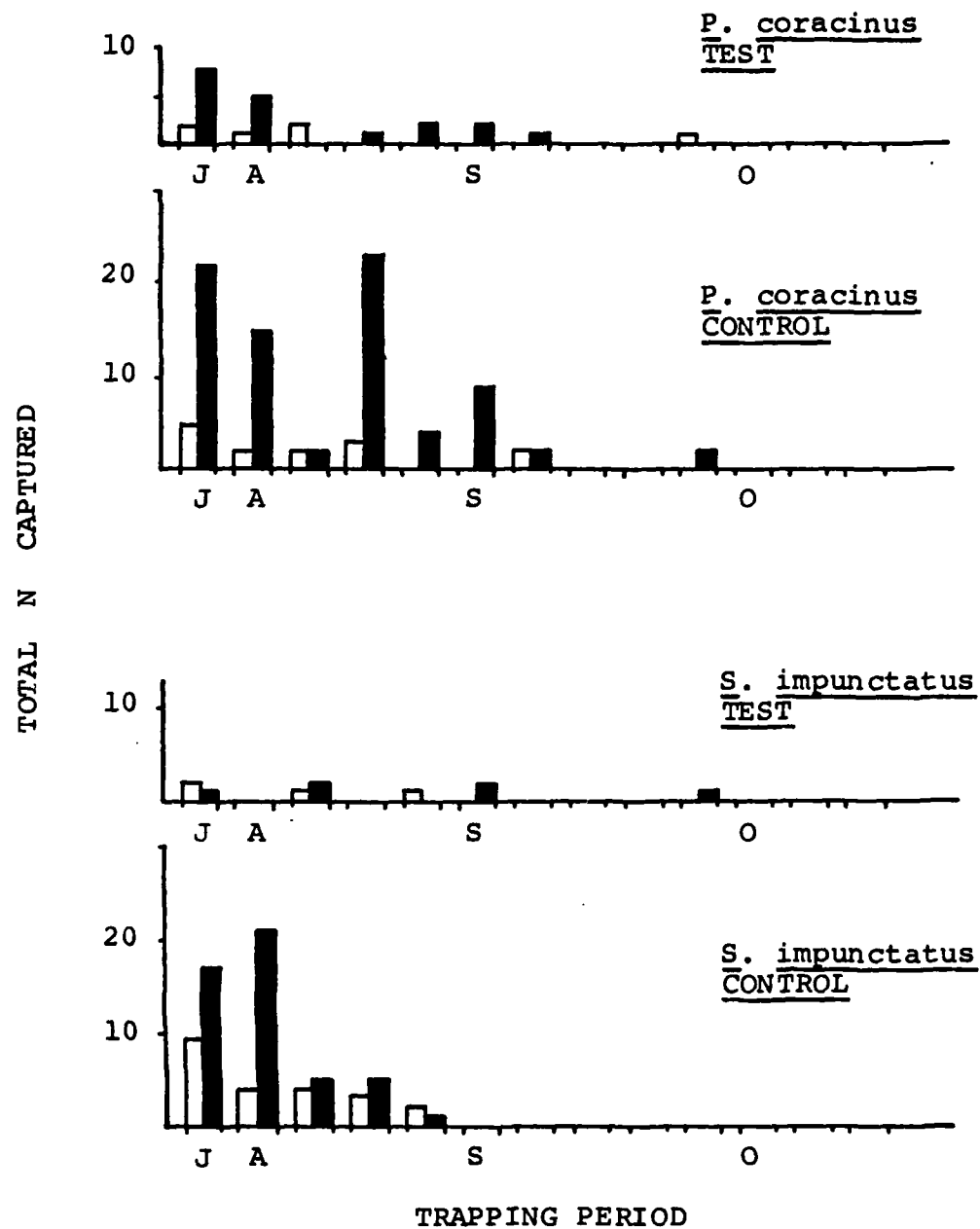


Fig. 34. Weekly diel activity of Pterostichus coracinus and Synuchus impunctatus in Test and Control, 1983 (open bars: day, black bars: night). For exact dates, see Fig. 30.

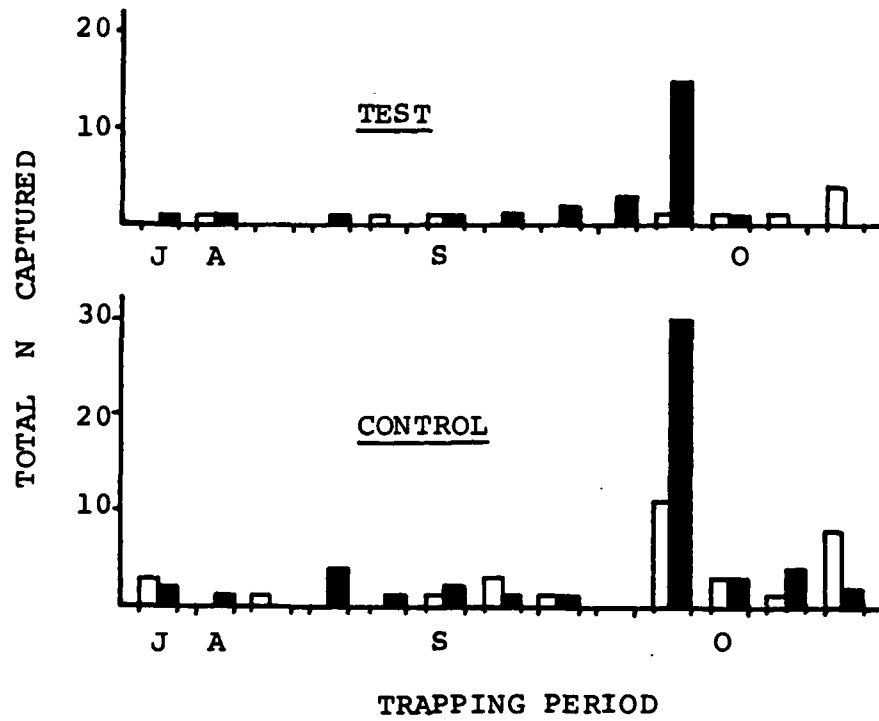


Fig. 35. Weekly diel catches (open bars: day, black bars: night) of Pterostichus pensylvanicus in Test and Control, 1983. For exact dates, see Fig. 30.

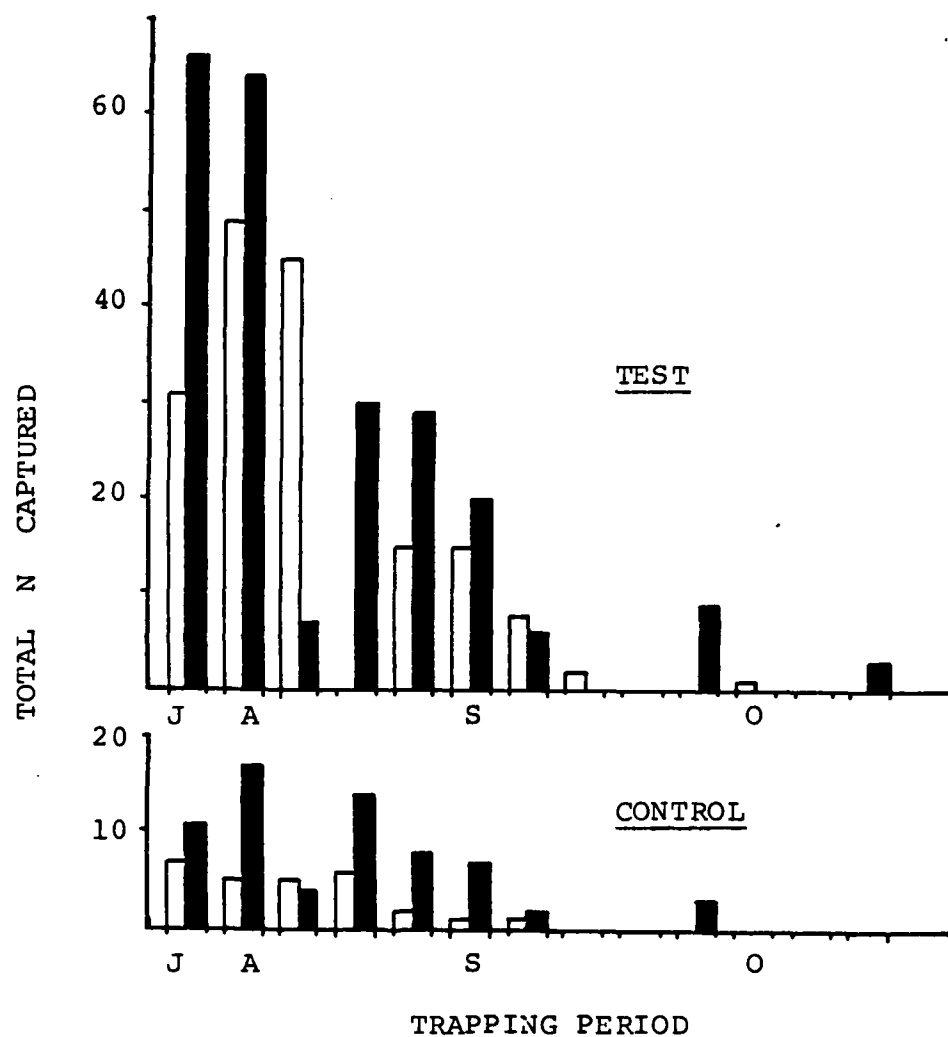


Fig. 36. Weekly diel catches (open bars: day, black bars: night) of Pterostichus melanarius, 1983. For exact dates, see Fig. 30.

Table 19. Number of species and individuals of Aranei captured in Test and Control, August to October 1983.

	N individuals	
	TEST	CONTROL
Linyphiidae		
<u>Bathypantes pallida</u> (Banks)	-	1
<u>Meioneta unimaculata</u> (Banks)	4	-
<u>Meioneta</u> sp.	-	3
<u>M. viaria</u> (Blackwall)	3	4
<u>Macrargus multesimus</u> (O.P. Cambridge)	-	2
<u>Centromerus sylvaticus</u> (Blackwall)	2	-
<u>C. persoluta</u> (O.P. Cambridge)	19	36
<u>Helophora insignis</u> (Blackwall)	2	6
Micryphantidae		
<u>Ceraticelus fissiceps</u> (O.P. Cambridge)	-	1
<u>C. laetabilis</u> (O.P. Cambridge)	-	8
<u>C. minutus</u> (Emerton)	-	2
<u>Ceratinopsis interpres</u> (O.P. Cambridge)	1	-
<u>Cornicularia directa</u> (O.P. Cambridge)	3	1
<u>C. minuta</u> Emerton	11	4
Amaurobiidae		
<u>Amaurobius borealis</u> Emerton	-	2
<u>Callobius bennetti</u> (Blackman)	-	1
Clubionidae		
<u>Clubiona canadensis</u> Emerton	4	-
<u>Phrurotimpus borealis</u> (Emerton)	10	-
<u>P. alarius</u> (Emerton)	1	-
<u>Agroeca ornata</u> Banks	5	28
Salticidae		
<u>Talavera minuta</u> Banks	4	-
Theridiidae		
<u>Mangora placida</u> (Heutz)	4	-
<u>Ctenium riparius</u> (Keyserling)	1	3
Hahniidae		
<u>Neoantistea agilis</u> Keyserling	1	5
Agelenidae		
<u>Agelenopsis utahana</u> (Chamberlin & Ivic)	6	5
<u>Cicurina robusta</u> Simon	5	18
<u>Wadotes calcaratus</u> (Keyserling)	1	8
Thomisidae		
<u>Oxyptila americana</u> Banks	14	14
<u>Xysticus elegans</u> Keyserling	7	1
Lycosidae		
<u>Lycosa gulosa</u> Walckenaer	5	3
<u>Trochosa pratensis</u> (Emerton)	3	5

In order to identify night- and day-activity in common species, we took recourse to specimens trapped at the Turner Road site (no longer used for the project after 1983, but similar to Test and Control): on a tentative basis, for species represented by >10 specimens total (three sites

combined), the diel assignments in Table 20 were made. All species listed are shared between sites, and many were relatively abundant in Test and Control in late 1983.

Table 20. Diel activity habits of spiders, in % of total catch (N) stemming from three sites, 1983.

SPECIES (N)	Nocturnal %	Diurnal %
<u>Helophora insignis</u> (13)	61.5	
<u>Agelenopsis utahana</u> (35)	68.6	
<u>Cicurina robusta</u> (39)	66.7	
<u>Wadotes calcaratus</u> (23)	82.6	
<u>Neoantistea agilis</u> (32)	71.9	
<u>Agroeca ornata</u> (64)	67.2	
<u>Ctenium riparius</u> (12)	66.7	
<u>Trochosa pratensis</u> (40)	57.5	
<u>Centromerus persoluta</u> (30)	46.7	53.3
<u>Oxyptila americana</u> (22)	44.5	54.5
<u>Cornicularia minuta</u> (27)		81.5
<u>Xysticus elegans</u> (11)		63.6
<u>Lycosa gulosa</u> (25)		72.0

Even considering that Table 19 only shows numbers trapped in late summer and fall, it is clear that this diverse group of predators cannot be analyzed at the species level without a significant increase in total individuals trapped. Once again, barrier data will provide some information on activity patterns under conditions in the Control site. 1984 data from both sites are not yet available at the species level; we suspect, however, that barrier-trapping in the coming 1985 season will be needed for a really useful data base, especially for moderately abundant species.

V. LUMBRICIDAE

Lumbricid population data for Test and Control are available from four dates in 1983 and two in 1984. Samples obtained on the remaining 10 dates in 1984 are currently being identified. Procedures for biomass estimation are detailed below; a manuscript (Pedobiologia, in press) dealing with the efficiency of our sampling techniques is appended (Appendix C).

1. METHODS:

1. Biomass estimation

As a rule, worms and cocoons are not weighed until the winter months, when the season's samples are processed. It was, therefore, necessary to quantify the relationship between live and preserved weights, given the specific handling and preserving methods we employ. Specimens obtained during handsorting of September 25 and October 15, 1984 samples were used for this purpose.

Protocol:

Individual worms were washed in water, briefly blotted dry, weighed and returned to clean water. Routine killing procedures were then applied: relaxation by gradually adding small amounts of alcohol to the water (concentration variable with size and species); brief, repeated rinsing with 95% ethyl alcohol, inducing contraction and death. The specimens were then fixed for several minutes in 95% alcohol, and preserved in 10% formalin.

Cocoons were rinsed in water, rolled on paper towel until dry, weighed, and preserved in 10% formalin.

All specimens were again blotted dry and weighed after 4-5 weeks.

Results:

More than 50% of the cocoons of five species, excluding Aporrectodea longa (Ude) because of low replication, underwent no weight change due to formalin preservation. The remainder showed both gains and losses (Table 21). Maximal change in both cases was 1 mg. Given the precision of the balance used, however, true gains and losses were within a range of 0.5 to 1.4 mg. Clearly (Table 21), weights of preserved cocoons are useable as fresh weights, without adjustments.

Table 21. Cocoon weight of five species: fresh weight ranges, and number of cocoons which remained unchanged, lost or gained after preservation.

	range,mg	N	no change	lost	gained
<u>Aporrectodea turgida</u> (Eisen)	8-17	35	23	8	4
<u>A. trapezoides</u> (Duges)	21-29	9	6	1	2
<u>A. tuberculata</u> (Eisen)	15-27	68	37	9	22
<u>Lumbricus rubellus</u> Hoffmeister	7-14	66	37	20	9
<u>Dendrobaena octaedra</u> * (Savigny)	34-39	30	19	5	6

* - all data are for groups of 10 cocoons

Regressions of fresh on preserved worm weights were highly significant (Table 22), and differed between species (f test, $P < 0.01$). Fresh biomass can thus be accurately estimated for all Test and Control species except D. octaedra. The species was obtained almost exclusively from leaf litter by formalin extraction, which made the specimens unsuitable for biomass validation. D. octaedra will be handcollected separately in spring of 1985 and processed as described above.

Table 22. Statistics for regression of fresh on preserved weight for five species of lumbricids (all F values highly significant).

	Regression equation	Expl.SS	Unexpl.SS	df	F
A.trapezoides	$Y=9.4924+1.0387X$	58614.1	341.3	10,1	861.78
A.turgida	$Y=0.6880+1.0497X$	21302.6	71.5	50,1	298.03
A.longa	$Y=-1.8176+1.0182X$	610101.6	286.5	10,1	2129.45
A.tuberculata	$Y=-0.7186+1.0538X$	72472.3	156.1	54,1	464.24
L.rubellus	$Y=1.5608+1.0214X$	46198.9	53.6	30,1	861.78

ii. Sample replication

In 1984, 10 samples were taken every two weeks, rather than 20 samples every three weeks as in 1983. Based on May and October 1984 data, we can tentatively evaluate the effect of the new sampling schedule on the accuracy of population estimates. In Table 23, coefficients of variation for each date are listed by species, omitting the sparse L. rubellus population in Control as well as low-density cocoon data.

With the exception of cocoons (D. octaedra, L. rubellus, and A. tuberculata), there is little evidence that a replication of 10 samples resulted in increased variability, given the inherently clumped (negative binomial) distribution of both worms and cocoons. Pending examination of all 1984 data, we have decided to retain the 1984 sampling schedule. Its advantage, over the 1983 schedule, is the shorter interval between samplings, which enhances interpretation of phenological data.

Table 23. V (coefficients of variation) of lumbricid samples in Test (T) and Control (C), for each date.

	DATE					
	1983 8/8	8/23	9/12	10/7	1984 5/7	10/15
<u>D. octaedra</u> (C)						
cocoons	67	54	43	55	80	79
worms	73	51	65	65	109	69
<u>D. octaedra</u> (T)						
cocoons	108	114	135	140	169	156
worms	148	108	127	100	129	121
<u>L. rubellus</u> (T)						
cocoons	80	78	85	81	122	125
worms	61	65	85	55	74	37
<u>A. turgida</u> (C)						
cocoons	109	151	107	147	172	99
worms	43	51	42	61	52	69
<u>A. trapezoides</u> (C)						
cocoons	182	-	-	-	-	108
worms	81	60	106	67	72	74
<u>A. tuberculata</u> (T)						
cocoons	97	100	-	-	-	185
worms	65	56	46	40	47	43
<u>A. longa</u> (T)						
worms	67	68	95	74	74	100

2. EARTHWORM POPULATIONS

Average densities and biomass, using data from all 6 dates, are given in Table 24. The Control site harbors a dense population of the small, litter-feeding D. octaedra; L. rubellus, a shallow-burrowing, robust species, which also lives in leaf litter if moist, is sparse. The same two epiges occur in Test, but are heavily dominated by L. rubellus. In both sites, a medium-sized endoge (A. tuberculata in Test, A. turgida in Control) dominates numerically and contributes most of the biomass. A. longa and A. trapezoides, large soil-dwelling species, furnish appreciable biomass but relatively few individuals (Table 24). Total lumbricid densities were approximately 3-400/m² in Test, and 4-500/m² in Control, mainly due to a large population of D. octaedra.

Lacking the bulk of 1984 data, presentation of 1983 data is not expedient at this time, and statistical analyses will not be performed until all samples are processed. However, in order to give an example of the nature of the data base, we will describe information obtained on D. octaedra, the dominant epige in Control.

Table 24. Density and biomass/m² (means of 6 dates \pm SE) of lumbricid populations in Test and Control; cocoons not included.

	TEST		CONTROL	
	N/m ²	g/m ²	N/m ²	g/m ²
<u>D. octaedra</u>	43.3 \pm 7.4	1.3 \pm 0.3	152.8 \pm 21.3	5.6 \pm 0.7
<u>L. rubellus</u>	71.2 \pm 5.4	8.8 \pm 1.5	4.4 \pm 1.3	0.9 \pm 0.4
<u>A. tuberculata</u>	243.2 \pm 15.6	59.3 \pm 10.4	-	-
<u>A. longa</u>	28.5 \pm 2.7	9.5 \pm 1.7	-	-
<u>A. turgida</u>	-	-	270.4 \pm 33.8	39.0 \pm 7.5
<u>A. trapezoides</u>	-	-	48.1 \pm 9.9	15.7 \pm 4.7

Phenology of *Dendrobaena octaedra*:

In both sites, immatures far outnumbered adults, beginning in late August (Fig. 37). On all dates, the majority of adults exhibited glandular clitella; the highest percent aclitellates occurred on August 8, in Control (19%). Cocoon densities (estimates \pm SE entered above bars in Fig. 37) reached a peak of >1300/m² in Control.

Because D. octaedra cocoons are semi-transparent, they could be assigned to developmental stages, distinguished visually, with no implication of relative chronological age:

"new": no pigmentation visible;

"intermediate": some pigmentation and segmentation visible, nutritive material still abundant in the cocoon;

"old": young worm completely formed and pigmented, no nutritive material left around it.

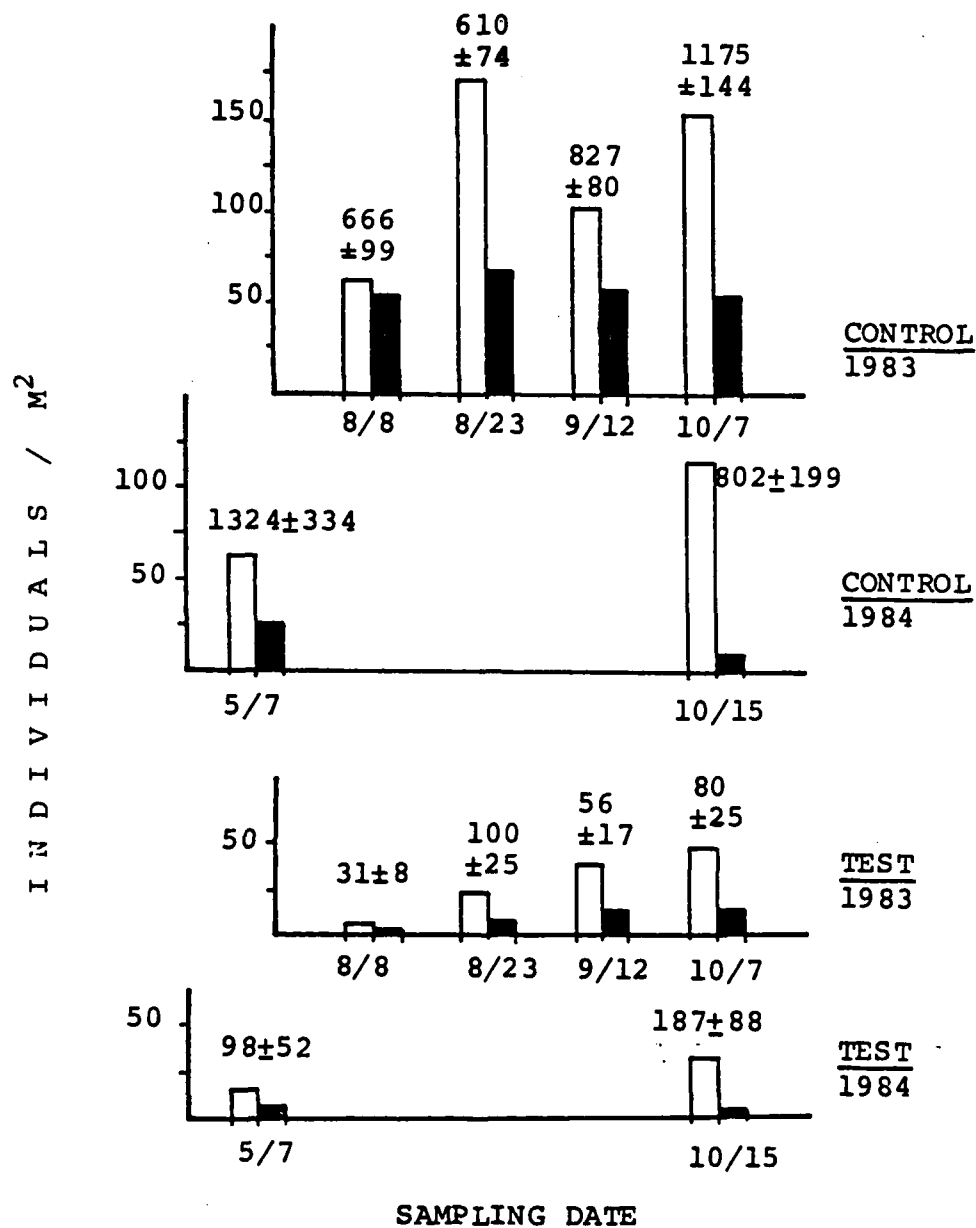


Fig. 37. Density/m² of adult (black bars) and immature (open bars) Dendrobaena octaedra in Test and Control. Cocoon densities ± SE written in above bars.

Relative abundance of cocoon stages, immature density/m², and average weight/individual, are combined in Fig. 38. Cocoon production in August seemed to proceed at a low, but constant rate in both sites. The main reproductive periods, however, occurred in spring and fall (ref. absolute numbers in Fig. 37).

Emergence of immatures may have contributed to relatively low mean weights in August and October (hatchlings weigh 3 to 4 mg). The converse, i.e., high mean weights due to absence of immatures, was more clearly expressed: in May, low immature density, high mean weights, and a preponderance of old cocoons coincided. The latter in particular indicated an imminent wave of recruitment. In mid-October 1984, new and intermediate cocoons were equally common. By the following spring, presumably, these intermediates would be fully developed and ready to hatch. Although immature weights did not reflect emergence well in the Test population, cocoon development was synchronous in both sites (Fig. 38).

Vertical distribution of D. octaedra showed the species to be restricted to the A and leaf litter layers. Less than 10% of all individuals, and frequently none, were recovered from B horizon samples on any given date.

Immatures and adults migrated into and out of the litter layer together, with some exception (October 1983, Fig. 39). Although the majority of cocoons are found in the A layer, reproducing adults frequent the litter if suitably moist. In May, for instance, at a time when 50% of all cocoons were new (Fig. 38) and production was high (Fig. 37), no adults were found in the A layer (Fig. 39). One cannot discount, however, a potential effect of time of day (and the climatic factors associated with it) when samples were taken.

For each species, we thus obtain seasonal densities, biomass and vertical distribution of all life stages, including cocoons. In final

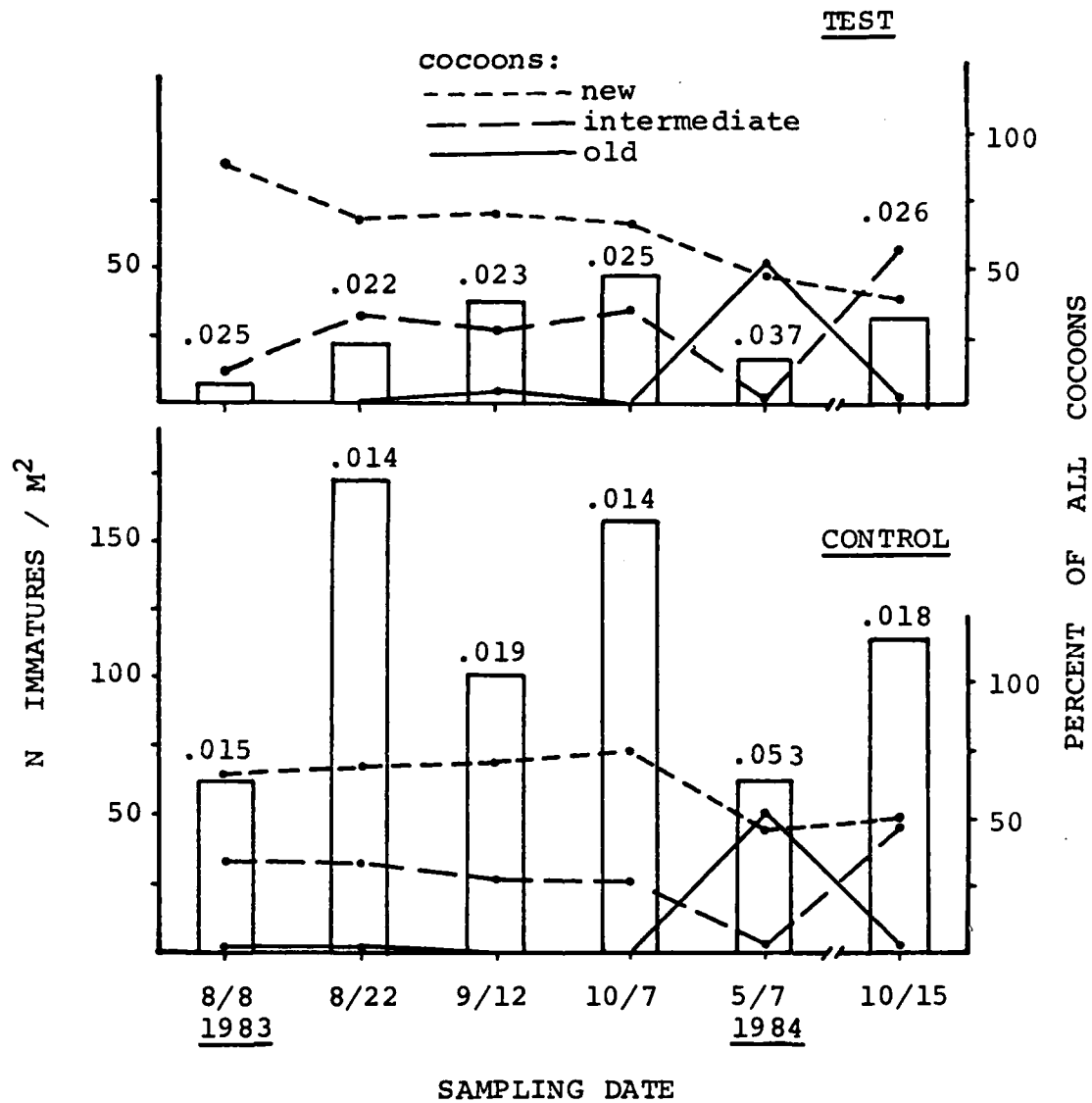


Fig. 38. Dendrobaena octaedra: density of immatures (bars), mean weight per individual (values above bars), and percent of cocoons in new, intermediate and old stages, in Test and Control.

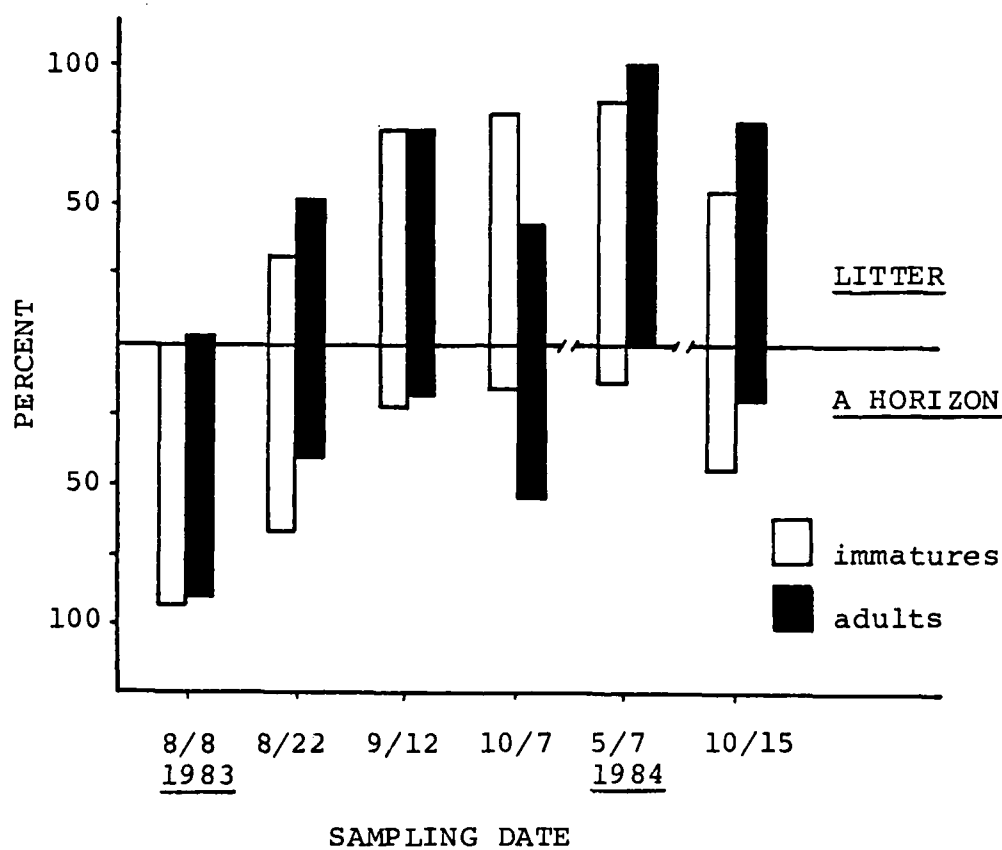


Fig. 39. Depth distribution of D. octaedra, in percent of individuals in all vertical layers.

analyses of density and distribution of lumbricid populations, environmental factors to be taken into account include: depth of the A horizon, litter mass estimates, litter and soil moisture, and soil temperature. Not all apply to all species (A. tuberculata, for instance, is absent from litter), so that variables will be selected for multiple regression as appropriate to each species' life style and phenology. More detailed analysis of weight frequency distribution (1983 and 1984) will be performed to obtain an estimate of development time and cohort growth in D. octaedra and other abundant Test and Control species. In addition, ANOVA will be used to determine whether weights of reproductive adults and of cocoons differ significantly as a function of time of year.

VI. LITTER DECOMPOSITION

Four principal study elements were expected to serve for process-oriented Test/Control comparison:

- a. litter input and elemental concentration of abscised leaves;
- b. seasonal litter standing crops;
- c. mass loss and elemental changes of confined litter, and identification of associated arthropods;
- d. mass and surface area losses of unconfined leaves.

Elemental analyses have not yet been returned by our Soil Testing Service, and we postpone reporting all pertinent data (1983 and 1984). In the following, we present available information, identify program shortcomings, and describe an alternative element implemented late in 1984.

1. METHODS

1. Worm litterbags:

In order to estimate a potential source of variation in decomposition studies, "worm bag" data were obtained in 1984, concurrently with litterbag sampling. Worm bags (1 mm litterbags in the field since November 1983) were brought in, worms were formalin-extracted from them, and the bags were refilled with litter which had also been in the field since 1983 (in litter reservoirs).

Control worm bags contained more animals than Test bags; relative occurrence of species (D. octaedra : L. rubellus, Table 25) reflected clearly the dominance of D. octaedra in Control (Table 24). Only three small immatures of A. tuberculata ever strayed into the bags.

D. octaedra was an opportunistic invader of litter given suitable moisture conditions (Table 25), more so than L. rubellus. The latter,

although dominant over D. octaedra in Test, invaded litterbags in relatively low numbers. Its apparent preference for the A horizon, together with its robust size, influenced its frequency in mesh bags.

In general, the number of worms/bag increased with time. If litter age (i.e., quality) was a factor determining invasion rate, then its effect was masked by low litter moisture, or compounded by high litter moisture, at the time of sampling (Table 25). May 8 samples were an unexplained exception: only one or two worms occupied the bags, at a time when litter was wet and almost 100% of the population at large occupied the leaf litter (Fig. 39).

Earthworms thus have the potential (varying with moisture fluctuation) of influencing confined litter breakdown to a different extent in Test and Control. Unless feeding rates and time of residence are known, the effect cannot be quantified. However, in view of litterbag decomposition data presented in section VI.4., it may not be relevant to data interpretation.

Table 25. Mean+SE lumbricids extracted from worm litterbags in Test and Control, relative species composition in terms of ratio D. octaedra : L. rubellus, and litter moisture (% of dry weight) at the time of sampling.

	SAMPLING DATE						
	5/8	6/5	7/5	7/30	8/27	9/25	10/15
TEST							
mean	0.17	0	1.50	0	8.08	8.33	4.58
SE	0.11	-	0.44	-	1.16	1.33	0.96
<u>octaedra:</u>							
<u> rubellus</u>	0:2	-	9:9	-	85:12	85:15	39:14
% moist.	134.8	11.0	42.3	15.5	95.8	176.3	92.8
CONTROL							
mean	0.25	0.42	11.60	0	18.30	10.10	24.00
SE	0.16	0.13	2.88	-	3.55	1.92	3.03
<u>octaedra:</u>							
<u> rubellus</u>	1:0	2:0	115:1	-	183:0	101:0	239:1
% moist.	148.7	10.4	117.6	11.1	103.5	186.6	100.7

ii. Arthropod extraction:

Arthropods were routinely heat-extracted from unopened litterbags to avoid loss of leaf material during handling. A potential disadvantage, curtailed extraction efficiency, was checked in September and October 1984: intact bags, as well as litter removed from bags, were heat-extracted in pairs. The members of each pair (with and without bags) had been in adjacent positions in the field.

Two conclusions were arrived at:

a. Litterbag litter had a high degree of integrity, so that loss of leaf material was virtually nil if removal was done with care;

b. Either method was appropriate for obtaining microarthropods, since average yields (Table 26) did not differ significantly. Test litterbags contained larger numbers of arthropods than Control bags. After partial breakdown into common groups, mites were shown to be responsible for the difference (Table 26). They were not only more abundant in Test bags, but highly aggregated: 73% of 3241 mites in intact bags came from 2 samples, and 57% of 2857 were extracted from another two samples without bags.

Table 26. Number of arthropods extracted from intact litterbags (w) and from litter removed from them (w/o). N = 16 for each site and sample type.

	TEST		CONTROL	
	w	w/o	w	w/o
Mean/sample	254.0	219.2	137.8	139.3
+SE	63.5	54.8	34.5	34.8
Range	32-1906	47-1016	36-257	51-432
N Acari	3241	2857	1336	1424
N Isotomidae	238	207	394	380
N Entomobryidae	404	285	320	295
Other Arthropoda	181	158	155	130

2. LITTER INPUT

Data are derived from 20 (0.25 m²) litter traps per site.

In 1983, leaves collected October 6 had accumulated since September 22 (2 weeks); peak litterfall in 1983, for both basswood and maple, occurred, therefore, prior to October 6. In 1984, peak abscission for maple occurred between October 7 and 15, about one week later than in 1983. Litter input declined abruptly during the following 7 days, unlike 1983 where the decline was gradual over approximately 3 weeks (Fig. 40).

The long sampling interval between August 25 and September 25, 1984 masked any differences in basswood litterfall between successive years. Minor species (Fig. 40), unlike the dominant maple, peaked earlier in 1984 than in 1983. Response of trees to climatic conditions, in terms of timing and rate of litterfall, was clearly identical in both sites, as were litter input amounts.

Cumulative litter input is illustrated in Fig. 41. Approximately 200 g/m² (maple) or 250-300 g/m² (all species) were added to the forest floor, with little variation between sites or years.

3. LITTER STANDING CROPS

Data were obtained from litter moisture samples after oven-drying at 60 C. One Control sample was accidentally destroyed (September 10, 1984).

Average standing crops are shown in Fig. 42. Control values were almost invariably higher than Test, and gave the impression of little breakdown from May to August. High estimates were obtained in June and August for Test litter mass, which declined through July. September lows (actual in Test, postulated for Control) (Fig. 42) were followed by a clear increase proportional to litter input: litterfall, completed for both basswood and

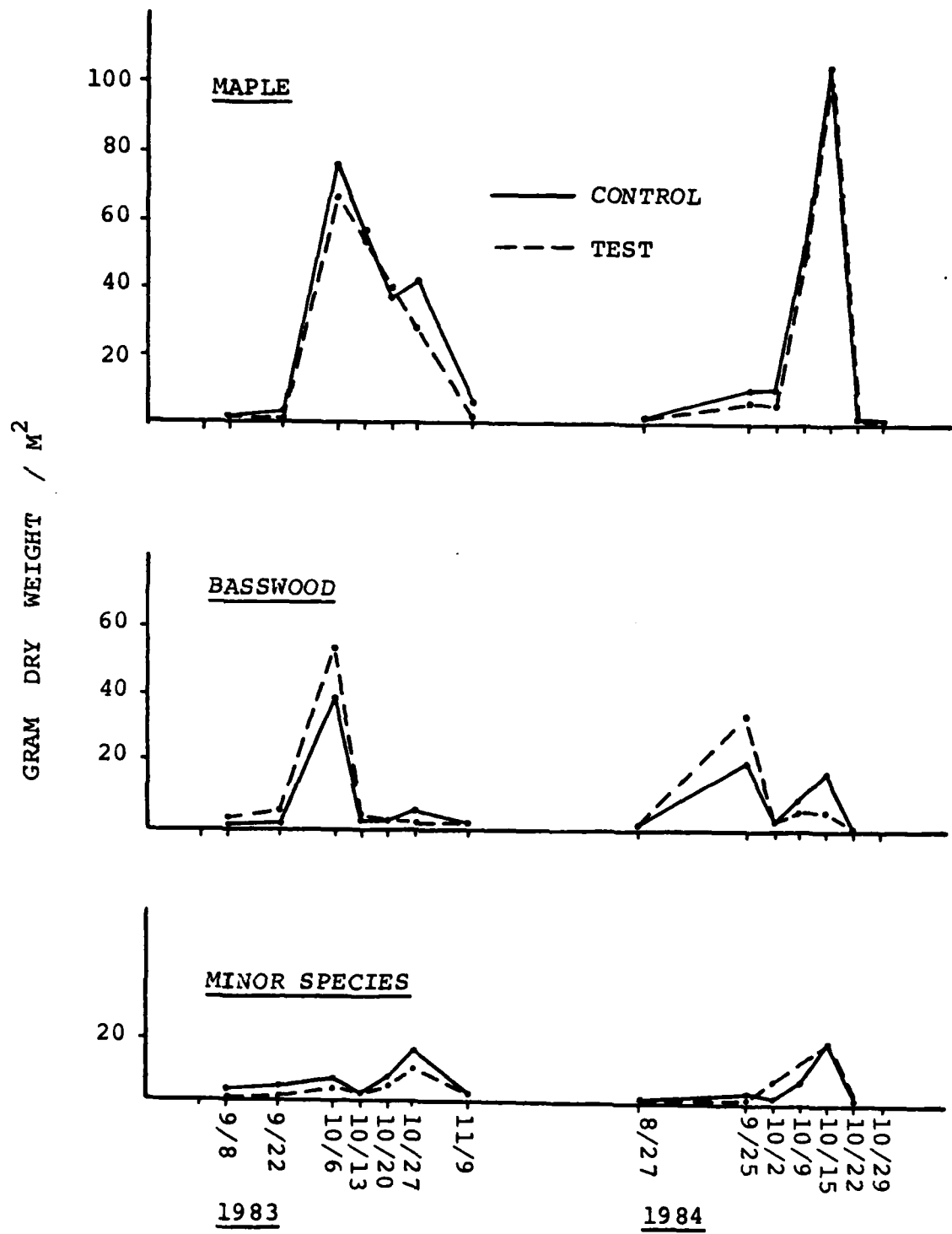


Fig. 40. Litter input (g dry weight/m²) in the fall of 1983 and 1984, Test and Control (SE omitted).

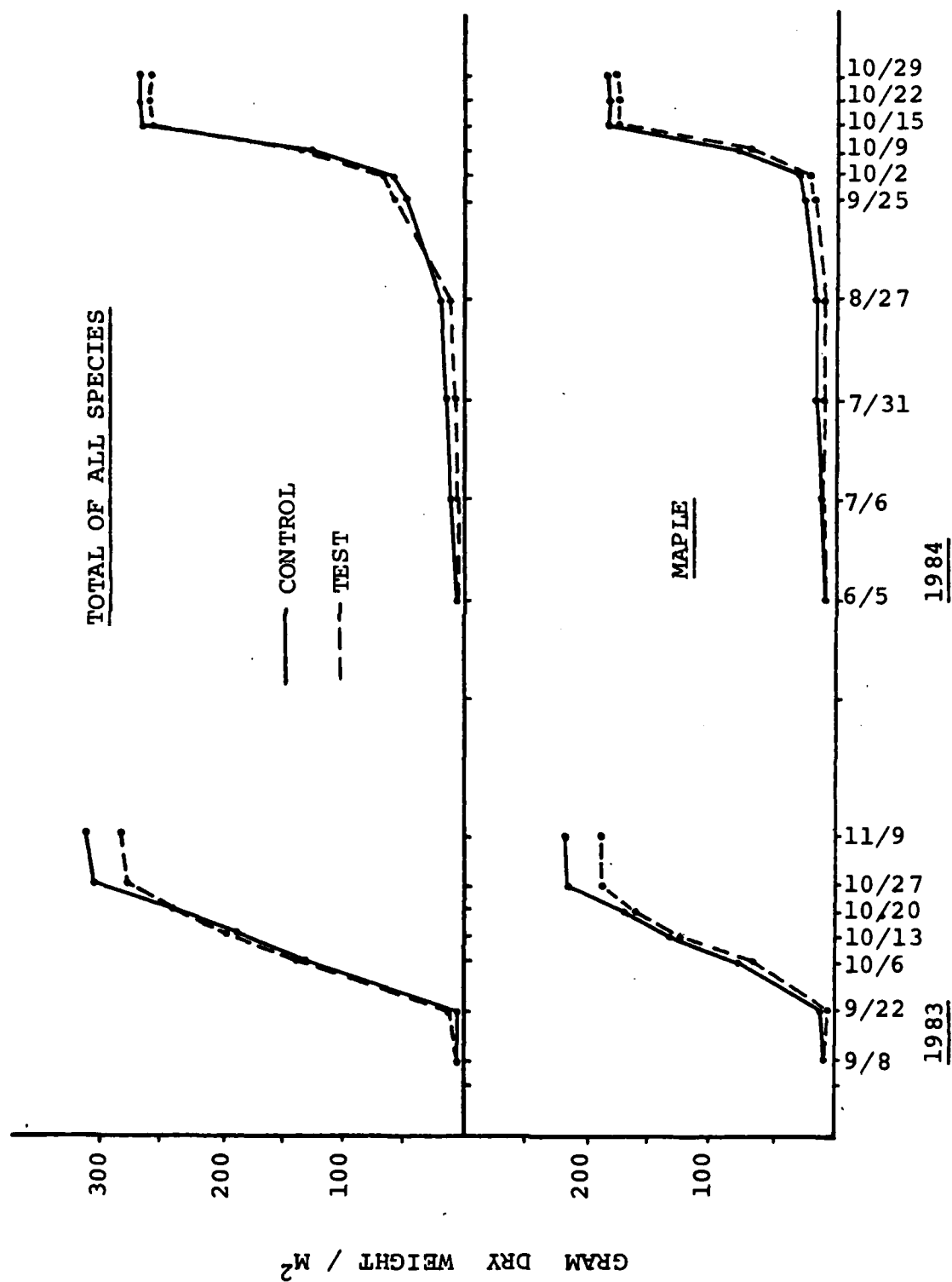


Fig. 41. Cumulative litterfall, g dry weight/m², in Test and Control, 1983 and 1984.

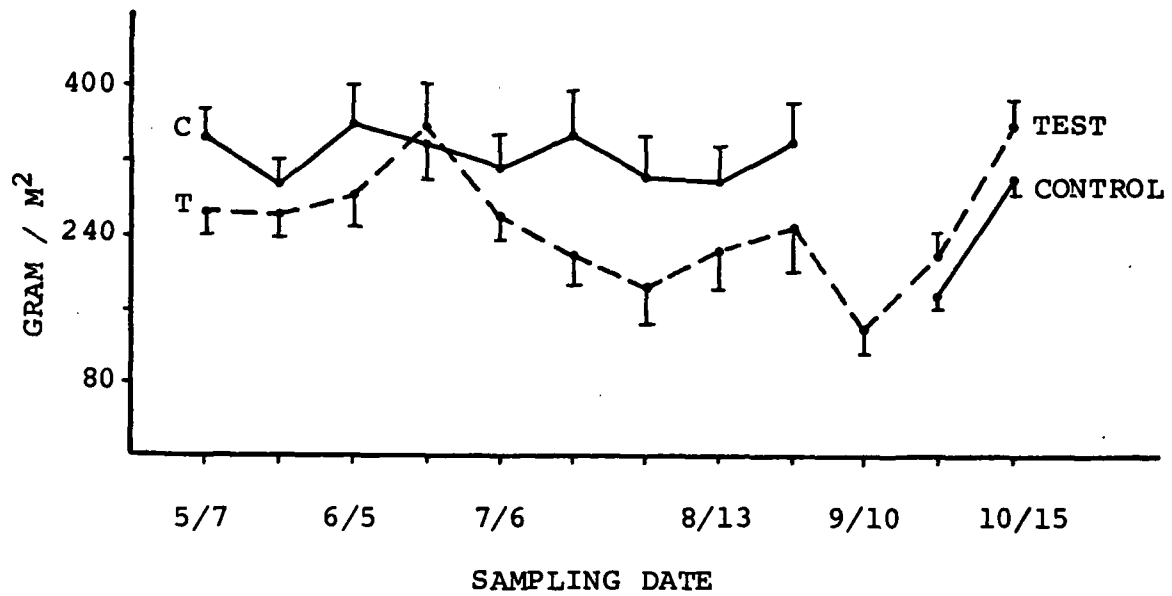


Fig. 42. Litter (all species) standing crops, 1984, in mean dry wts \pm SE. Samples taken at two-week intervals; the first date in each month identified exactly.

maple by October 15, added about 200 g/m² to the forest floor.

We are using several means to obtain mass and nutrient input and loss rates (standing crops, litterbags, unconfined leaves). Among them, standing crop estimates proved the most variable.

We can increase neither replication nor sample size (1/16 m²) for fear of excessive removal of substrate and site disturbance. We plan, therefore, to continue the present program (needed for moisture determination as well), but propose to supplement it with larger samples (1/4 m²), taken beyond the perimeter of Test and Control, but within the site sensu latu. Samples will be taken along linear transects, at constant intervals. Sample dates are chosen according to expected seasonal extremes in litter mass: early spring (over-winter loss estimates); just before leaf-fall (maximal yearly loss); and after leaf-fall is complete (maximal yearly mass).

4. CONFINED LITTER DECOMPOSITION

In November 1983, over 100 (1 mm mesh) bags containing approximately 8-12 g maple litter were placed in Test and Control. Groups of 8 bags/site were retrieved on 6 dates in 1984, heat-extracted, and weighed when completely dry.

Confined litter lost approximately 15-20% of its initial weight between November 1983 and June 1984. From June to October, changes were minor and variable, but Control litter consistently retained higher weights than Test litter (Fig. 43), significantly so July 5 ($P < 0.05$) and July 30 ($P < 0.005$). If these are true site differences, then the activity of Dendrobaena octaedra (section VI.1.1.) did not increase decomposition rates as might have been expected.

Occasional litter weight gains were recorded, which is not uncommon in litterbag studies. During the entire first year, approximately 20% weight

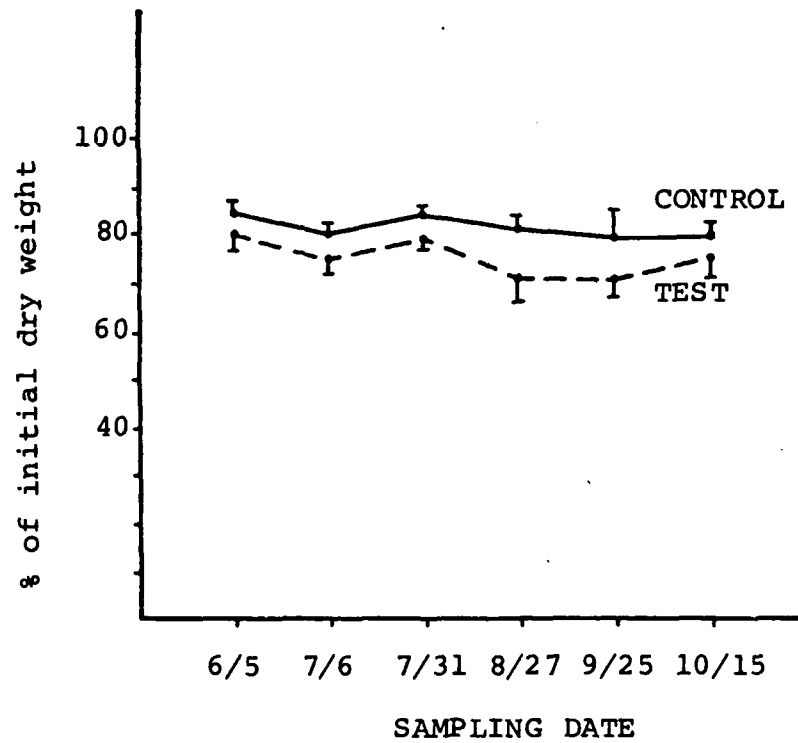


Fig. 43. Litter dry weights remaining in litterbags in the field since November 1983 (means \pm SE).

loss occurred in Control, and 25% in Test (Fig. 43). Although this appears to be consistent with higher standing crops in Control (Fig. 42), we are still reviewing techniques to eliminate human error in 1985. Unfortunately, Test and Control have to be sampled by two different teams due to the distance between sites and the amount of work to be done in one day.

No further analyses have been performed at this time, pending elemental analyses and decomposition data after the second winter in the field (1984-85). We expect to calculate exponential loss rates for the two sites by regressing weight remaining on time of exposure in the field, and to test the regressions for homogeneity.

In 1984, we considered using smaller mesh sizes (24 microns) in order to exclude all lumbricids. The tightness of the mesh, together with the quality of the nylon material we were testing, were found to prevent or retard passage of water. Finally, a duplicate series of 1 mm bags, and a new series of large-mesh (5 mm) bags were manufactured and put out in November 1984. A total of 120 bags/mesh size/site will provide for increased replication (10 bags/date, 12 dates through September of 1986).

With respect to the decomposer fauna, the only data we have are those of the extraction experiment (Table 26). We expect Test/Control litterbag faunas to be highly discrepant. Furthermore, we are concerned over the time and manpower needed if we are to identify the material to genus or species level. For the sake of continuity, we will extract arthropods from litterbags in 1985 and 1986. The material will, however, only be broken down to major groups (order, and family for selected orders), to obtain site-specific arthropod densities in litter of known age and moisture content.

5. DECOMPOSITION OF TROTLINE LEAVES, 1983-84

At leaf fall time, abscised maple leaves from each site were pressed until dry, weighed, run through a Licor area meter and xeroxed. Each leaf was identified by a numbered aluminum foil strip wrapped loosely around its petiole. Trotlines were made by knotting braided nylon line around leaf petioles, approximately 25 cm apart, 5 leaves per line, 42 lines per site. In the field, sets of 4-5 lines were attached to stakes and fanned out at 40-45 degree angles of each other.

At monthly intervals, 4-6 trotlines were retrieved by dissecting the leaves gently out of surrounding litter. They were rinsed, pressed, xeroxed, and area and weight were again recorded.

General results:

It soon became obvious that the trotline technique was not successful, due to the unexpected fragility of maple leaf petioles. In both sites, less than 30% of all leaves were recoverable, the rest were no longer attached to the strung-up stems. As a result, replication was poor and the experiment was discontinued at the end of the first year. One sampling date in Control was omitted entirely, to save remaining leaves for one last fall sample on September 26.

Leaf petioles also biased weight records: up to 50% of individual leaf weight (September 26 data) was contributed by the stem - obviously, the less leaf lamina left, the greater the bias. On the other hand, petioles also broke off easily after the samples were brought in, getting lost during the various manipulations.

During sampling, we observed that position of leaves relative to surrounding natural litter seemed to affect the degree of decomposition: those buried under litter drifts tended to be whole, though skeletonized, and were, of course, moister.

Weight and surface area changes:

May through August, remaining dry weights and surface areas did not differ significantly in Test and Control (Fig. 44). Variances increased in August and September, particularly for area measurements. We believe that leaf type (sun, shade), interacting with leaf position in natural litter, caused increasing variability as the season progressed. Because replication was much less than half of what we had planned, these effects could not be adequately tested. As an example, Fig. 45 shows three leaves from the September 26 Test sample: leaf 6 retained 98% of its area and 79% of its weight; leaf 21 retained 49% of its weight, and leaf 211 29%. Number 6 was a sun leaf (initially small and heavy), leaves 21 and 211 were not.

By late September, Control leaves had lost significantly more weight and area than Test leaves (Fig. 44). Unfortunately, the experiment could not be followed beyond this time.

Even with low replication, which magnified known (leaf type) and unknown sources of error, unconfined leaf breakdown seems to reflect site-specific processes well. This type of study is thought to be valid, because the data are unbiased by "litterbag effects". Analyses should be sensitive to changes once variation is further reduced by taking into account known sources of error.

We initiated a program in November 1984 which accomodates leaf type and position, while keeping as close to the concept of unconfined leaf decomposition as possible.

6. LEAFPACK STUDY, 1984-85

From maple litter collected in bulk during leaffall, three groups of leaves were sorted out:

A: Sun leaves, typically thick and leathery, of small size and dark

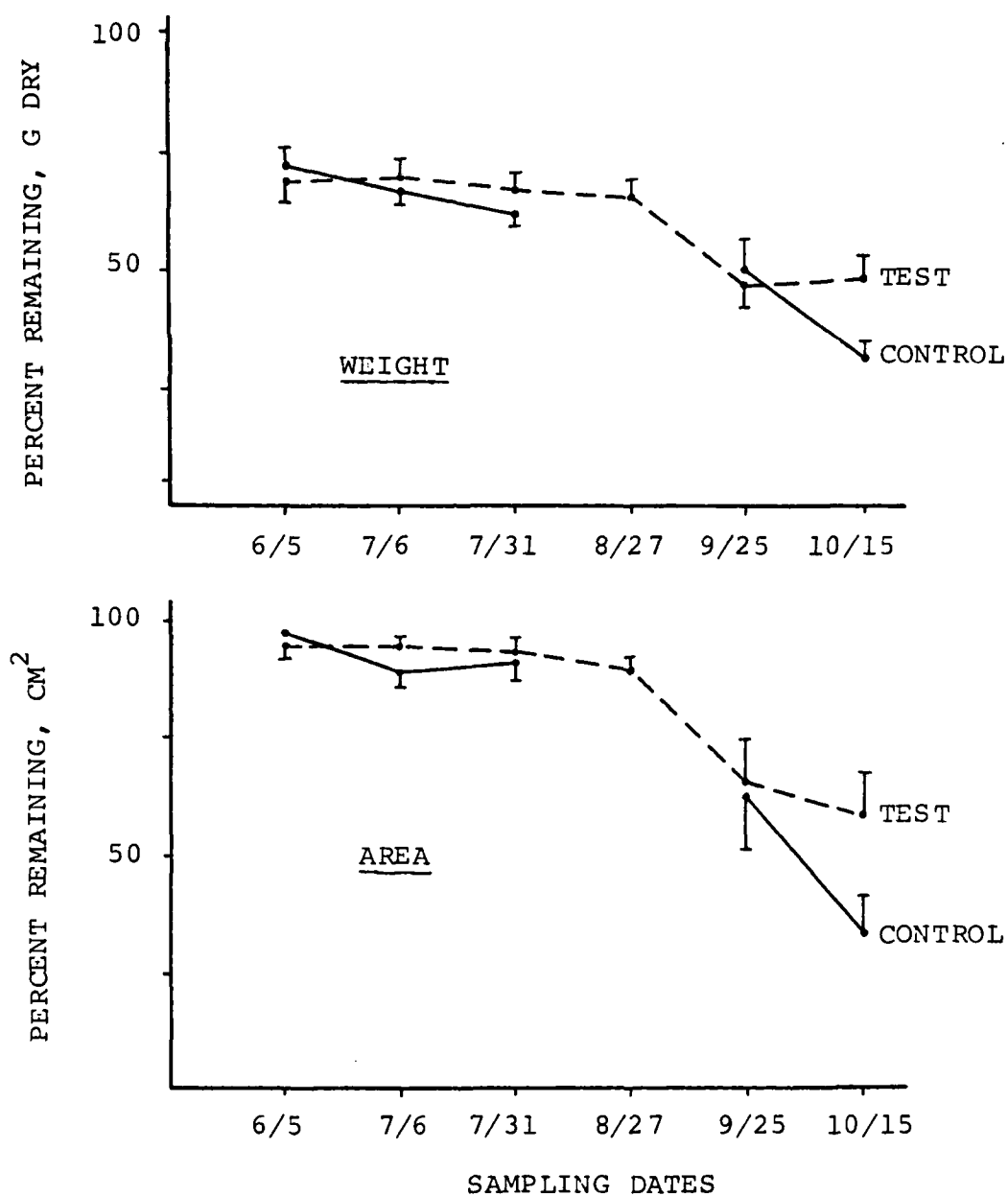


Fig. 44. Mean \pm SE remaining weights and areas for trotline leaves in the field since November 1983. (Replication variable from 5 to 12).

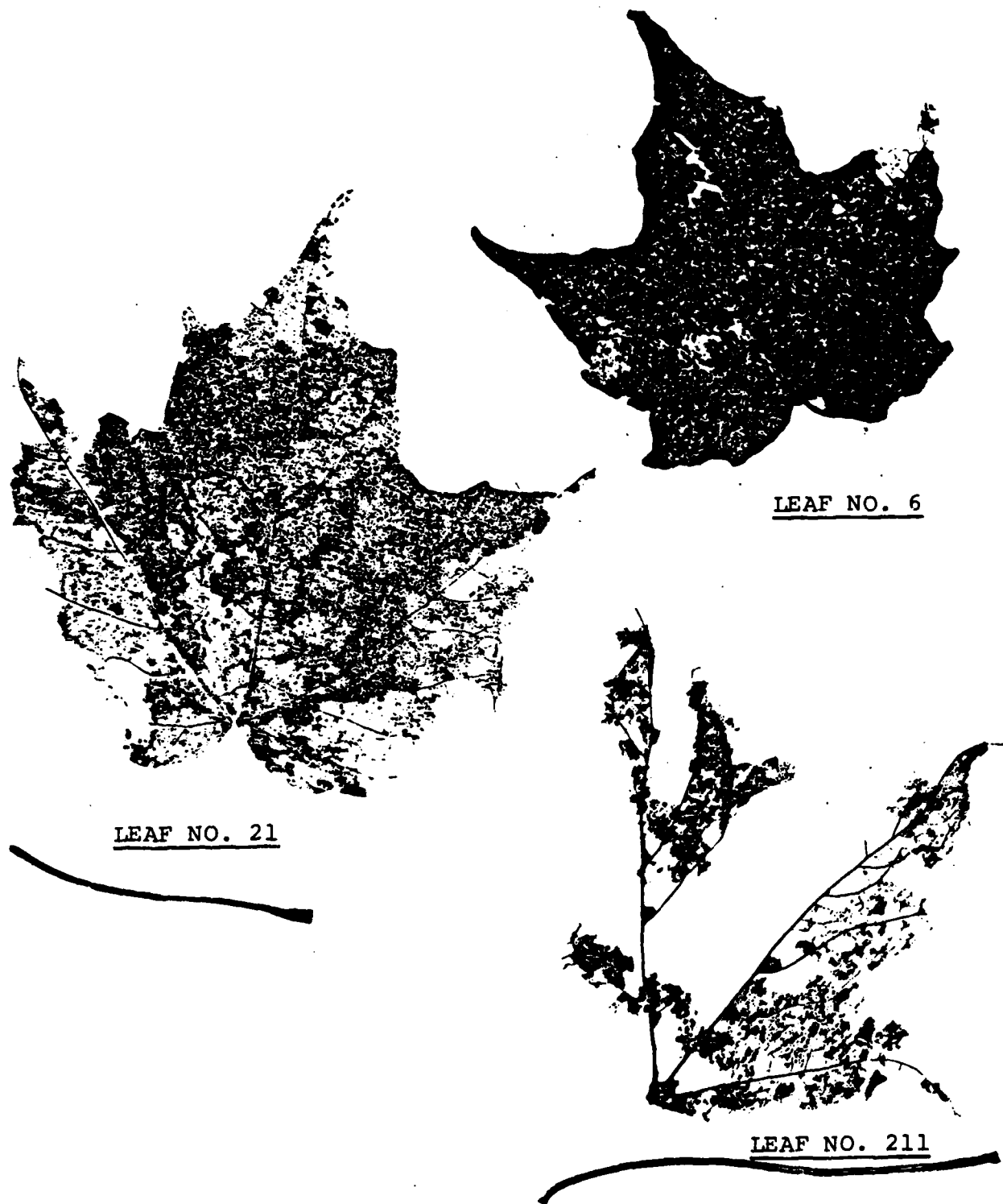


Fig. 45. Examples of trotline leaf remains after 11 months in the field (Test site, September 25, 1984).

color:

R: Shade leaves, typically large, thin, and pale yellow in color:

C: randomly selected mixed leaves, sun, shade, and intermediate in unknown proportions.

Leaves were pressed until dry. After breaking off the petioles, they were xeroxed, weighed, and their surface areas were recorded.

In packs of six of each group, they were taken into the field and positioned so that the bottom leaf rested directly on the soil surface. Each pack was covered by a loose hood of thin, flexible nylon netting (2.5 cm mesh size), secured by wooden pins pushed into the soil. Precise records ensured that position of each pack within the site, and position of each leaf within a pack, were known.

Sampling plans:

Sun and shade leafpacks will be retrieved 4 times during 1985, and twice in 1986; effects of vertical distribution within packs will thus be quantified for the two extreme types. Mixed leaves will be sampled at the rate of 8 packs at monthly intervals. Leaves are distributed randomly within these packs, mimicking natural conditions. In order to analyze their breakdown patterns, two factors must be considered: vertical position, which is known, and leaf type. The latter can be determined using weight and surface area, as discussed below.

Leaf type classification:

Sun and shade leaves were selected according to subjective visual and tactile criteria, and processed as fast as possible because weather conditions were worsening. Later, objective means of classifying leaves were sought, and the ratio of surface area/weight was found appropriate.

Sun leaves are characterized by high weights and small surface area, and vice versa for shade leaves. Within each leaf type, weight and area are

highly correlated ($P < 0.001$), with a coefficient r of 0.79 for sun and 0.92 for shade leaves.

Basic leaf type statistics, by site, are given in Table 27. For unknown reasons, site-specific leaf weights differed significantly in all categories, being higher in those collected from Test. Mixed Test leaves also had smaller surface areas and area/weight ratios, indicating that this group contained a larger proportion of sun leaves.

Area/weight ratios for sun and shade leaves (Table 27) differed significantly at $P < 0.0005$ for both sites, i.e. were good indicators of leaf type. Frequency distributions, not available at this time, will determine upper and lower limits of type classes; each leaf in mixed packs can then be ranked on a type scale, which will be used as factor in analysis of variance of decomposition rates.

Table 27. Mean (\pm SE) g dry weight, cm² surface area, and area/weight ratios for leafpack leaves collected in Test and Control, fall 1984.

	TEST		CONTROL		P
SUN LEAVES					
weight	0.388	0.010	0.330	0.007	.001
area	55.34	1.08	52.34	0.99	NS
a/w	165.84	12.62	178.48	11.04	NS
SHADE LEAVES					
weight	0.246	0.005	0.227	0.005	.01
area	83.04	1.54	79.73	1.72	NS
a/w	388.42	25.99	425.08	34.13	NS
MIXED LEAVES					
weight	0.263	0.006	0.198	0.004	.001
area	43.75	0.78	47.11	0.71	.01
a/w	193.67	7.69	267.47	9.14	.001

VII. CONCLUSION

We now have a good estimate of manpower required to accomplish each task, in the field as well as in the laboratory. Based on this estimate, and on preliminary Test/Control data, some re-direction of effort and re-definition of objectives are indicated. Changes, some of which were already implemented in 1984, are listed below:

1. Arthropod populations:

We will resume the 1984 sampling schedule in spring of 1985, with 20 samples taken, and 10 (all even-numbered ones) sorted. A review of time spent on taxonomic work, and of the nature of the data so far examined, have led us to re-allocate effort: rather than trying to identify all material to family level or below, we chose a few abundant groups shared between sites, specifically where Acari are concerned. Details of how the material will be processed are still being developed. In general, we believe that project goals are better served by specific data bases, as opposed to broad, insensitive trends in "total numbers" (above generic level).

Analysis of seasonal dynamics of "species complex A" (Mesostigmata), for instance, may require rearing its members to establish a reference collection of immatures. In Collembola, the common species should be categorized by size classes, so that recruitment and seasonal population structure can be made apparent. We feel that these kinds of selective goals justify an investment of time at the detriment of analysis of all taxa in semi-detail.

2. Pit-trapping:

Barrier traps without funnel inserts will be installed in spring of 1985. Twenty diel samples per week will be taken, as before, and the number to be sorted and identified will be determined by 1984 data (remaining

material stored).

3. Lumbricidae:

Ten samples are taken per site, in all even-numbered quadrats. D. octaedra biomass estimation is yet to be done. All methods remain as before.

4. Environmental monitoring:

With slight changes in methods (e.g., A and B horizon samples increased in volume), litter and soil sampling for moisture determination will be continued.

In 1985, existing rain gauges will be replaced with recording gauges, to pinpoint minor rainfall events which may affect arthropod surface-activity in each site (because of the distance to the Test site, readings were only taken on sampling days).

Throughout 1984, we experienced frequent problems, often undetected for long periods of time, with OMNIDATA remote sensing equipment. Assessment of the extent of the damage done to the data base is not yet complete. We know, for instance, that soil-litter interface temperatures for 1984 are probably not reliable. To help interpret data on litter-dwelling and surface-active species, we will have to take recourse to Weather Service records from several km away. In order to forestall future information gaps, we are currently investigating other types of sensing/recording devices (including a main and a backup system), to be purchased and installed in spring of 1985.

5. Litter turnover:

Objectives dealing with decomposition and elemental flux were first reinstated by us (after their deletion in the negotiated 1982 subcontract) in the fall of 1983. From November 1983 through 1984, we made time, and re-allocated funds, for as many sampling programs as we could accommodate.

The first season (1984) proved that these sampling and extraction schedules were not overly labor-intensive, except for identification of litterbag arthropods beyond the family level. Based on preliminary data discussed earlier, we here finalize our plans for this group of objectives.

Litter input will be monitored as before (20 traps); the switch from monthly to weekly collections of litterfall will be done earlier in the season, to fine-tune yearly differences in the onset of litterfall.

Standing crop estimates, derived from 20 (1/16 m²) samples, will be supplemented by additional samples from the site peripheries, on 3 dates per year.

Litterbag sampling, using 1 mm and 5 mm mesh bags, will continue on a monthly basis, beginning as soon as the sites are accessible. Arthropods extracted from them will be identified to order, selected orders to family. Because of large expected differences in species composition and densities, further breakdown would not serve future site comparison in proportion to the effort required.

Trotlines have already been replaced by leafpacks, designed to eliminate sources of variation in unconfined litter decomposition.

APPENDIX A

GROUND COVER IN TEST AND CONTROL

Appendix A: Frequency of occurrence of abundance values for ground cover species in Test (T) and Control (C).

	Abundance Value									
Species	0		1		2		3		Total	
	C	T	C	T	C	T	C	T	C	T
<u>Maianthemum canadense</u> Desf.	.23	.41	.49	.28	.19	.11	-	-	.91	.70
Sedge spp.	.12	.19	.57	.61	.26	.08	.01	.04	.96	.92
<u>Actaea alba</u> (L.) Mill.	.14	.78	-	.11	-	-	-	-	.14	.89
<u>Osmorhiza claytonii</u> (Michaux) Clarke	.78	.72	-	.16	-	.03	-	-	.78	.91
<u>Acer saccharum</u> Marsh.	.65	.58	.01	.23	-	.06	-	-	.66	.87
<u>Polygonatum commutatum</u> (R.& S.) Dietr.	.42	.71	.42	.19	.07	.01	.01	-	.92	.91
<u>Botrychium virginianum</u> (L.) Sw.	.79	.86	.01	.01	-	-	-	-	.58	.76
<u>Uvularia perfoliata</u> L.	.34	.14	-	-	-	-	-	-	.34	.14
<u>Populus</u> spp.	.33	.54	-	-	-	-	-	-	.33	.54
<u>Taraxacum</u> spp.	.58	.76	-	-	-	-	-	-	.58	.76
<u>Sanguinaria canadensis</u> L.	.14	.05	-	-	-	-	-	-	.14	.05
<u>Viola pubescens</u> Ait.	.37	.56	.46	.20	.05	.02	-	-	.88	.78
<u>Aster macrophyllus</u> L.	.06	.36	-	.01	-	-	-	-	.06	.37
<u>Schizacne purpurascens</u> (Torr.) Swallen	.11	.07	.08	.01	-	.02	-	-	.19	.10
<u>Arisaema triphyllum</u> (L.) Torr.	.08	.36	-	-	-	-	-	-	.08	.36
<u>Ostrya virginiana</u> (Mill.) Willd.	.35	.30	.01	-	-	-	-	-	.36	.30
<u>Amelanchier</u> spp.	.12	.10	-	-	-	-	-	-	.12	.10
<u>Oxalis</u> spp.	.01	.03	-	-	-	-	-	-	.01	.03
<u>Ulmus americana</u> L.	.02	.04	-	.	-	-	-	-	.02	.04

<u>Ribes</u> spp.	.31	.61	-	-	-	-	-	-	.31	.61
<u>Abies balsamea</u> (L.) Mill.	.25	.40	-	-	-	-	-	-	.25	.40
<u>Dirca palustris</u> L.	.41	.28	-	-	-	-	-	-	.41	.28
<u>Trillium grandiflorum</u> (Michx.) Salisb.	.61	.52	-	-	-	-	-	-	.61	.52
<u>Oryzopsis racemosa</u> (J. E. Smith) Ricker	.38	-	.04	-	-	-	-	-	.42	-
<u>Corallorrhiza</u> <u>corallorrhiza</u> (L.) Karst.	.07	.04	-	-	-	-	-	-	.07	.04
<u>Aralia racemosa</u> L.	.19	.38	-	.02	-	-	-	-	.19	.40
Polypodiaceae sp. 1	.18	.13	.04	.02	-	-	-	-	.22	.15
<u>Prunus serotina</u> Erhr.	.23	.74	-	.01	-	-	-	-	.23	.75
<u>Cornus</u> spp.	.22	.52	-	-	-	-	-	-	.22	.52
<u>Hieracium</u> spp.	.25	.51	.01	.07	.01	-	-	-	.27	.58
<u>Hepatica acutiloba</u> DC.	.01	-	-	-	-	-	-	-	.01	-
<u>Trientalis borealis</u> Raf.	.30	.05	.10	.01	-	-	-	-	.40	.06
<u>Clintonia</u> spp.	.08	.08	-	-	-	-	-	-	.08	.08
<u>Lycopodium obscurum</u> L.	.12	.02	.01	-	-	-	-	-	.13	.02
<u>Geranium maculatum</u> L.	.02	.03	-	-	-	-	-	-	.02	.03
<u>Aquilegia canadensis</u> L.	.03	.06	-	-	-	-	-	-	.03	.06
<u>Equisetum</u> spp.	.06	-	-	-	-	-	-	-	.06	-
<u>Galium triflorum</u> Michx.	.02	.75	-	.01	-	-	-	-	.02	.76
<u>Anemone quinquefolia</u> L.	.01	-	-	-	-	-	-	-	.01	-
<u>Erythronium americanum</u> Ker.	-	.21	-	-	-	-	-	-	-	.21
<u>Corylus cornuta</u> Marsh.	-	.31	-	.01	-	-	-	-	-	.32
<u>Rubus</u> spp.	-	.03	-	-	-	-	-	-	-	.03
<u>Fraxinus</u> spp.	.23	-	-	-	-	-	-	-	.23	-
<u>Sambucus</u> spp.	-	.07	-	-	-	-	-	-	-	.07
Polypodiaceae sp. 2	-	.10	-	.01	-	-	-	-	-	.11

<u>Caulophyllum</u> <u>thalictroides</u> (L.) Michx.	-	.10	-	-	-	-	-	-	.10
<u>Agrostis</u> spp.	-	.25	-	.16	-	.01	-	-	.42
<u>Claytonia virginica</u> L.	-	.01	-	-	-	-	-	-	.01
<u>Rubus parviflorus</u> Nutt.	-	.02	-	-	-	-	-	-	.02
<u>Tilia americana</u> L.	-	.02	-	-	-	-	-	-	.02
<u>Clematis verticillaris</u> DC.	.01	-	.01	-	-	-	-	-	.02
<u>Antennaria</u> spp.	-	.04	-	-	-	-	-	-	.04
<u>Stellaria</u> spp.	-	.01	-	-	-	-	-	-	.01

APPENDIX B

MANUSCRIPT 1, submitted to
The Great Lakes Entomologist

EVALUATION OF PIT-TRAP TRANSECTS WITH VARIED TRAP SPACING IN
A NORTHERN MICHIGAN FOREST (1)

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and

Richard J. Snider(2)

(1) Support for this research was provided by the Naval Electronic Systems Command through a subcontract to IIT Research Institute under contract N00039-81-C-0357

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ABSTRACT

The study compared effects of four distances between traps (range 0.5 to 4.0 m) on arthropod captures. Twelve traps were aligned in each of four transects, and 20 samples/trap were obtained during summer and fall, in deciduous forest in northern Michigan. Catches proved to be unaffected by trap spacing. Rather, they reflected local within-site differences in abundance of dominant species.

INTRODUCTION

In August 1982, we began preparing for a long-term investigation of forest-floor arthropods in Michigan's Upper Peninsula. Knowing that pit-trapping would be one of our research tools, we used the first half-season for a preliminary trapping experiment in hardwood forest. We intended to obtain taxonomic information on arthropods of the area, which is faunistically poorly described, as well as to quantify potential effects of different distances between traps on catch sizes.

SITE AND CLIMATE

The site was located in an extensive deciduous forest in Dickinson County (T 44 N.-R 29 W.-S 19), in the south-central portion of Michigan's Upper Peninsula. It was dominated by Populus grandidentata Michx. (55%), with Acer saccharum Marsh subdominant (34%). Amelanchier canadensis (L.) Medic. dominated the understory. Saplings and seedlings of Picea mariana (Mill.) and Abies balsamea (L.) Mill. were rare. Herbaceous vegetation was dense and even, with Pteridium aquilinum (L.) Kuhn, Lycopodium obscurum L. and Aster macrophyllus L. its most conspicuous components in mid- and late summer. Typical of this once glaciated region, the site consisted of an elongate ridge flanked by shallow depressions.

The area has a temperate continental climate of the cool summer type (30-year average temperatures for July: 26 C maximum, 3 to 4 C minimum). Annual normal precipitation is 76 cm, evenly distributed, with snowfall occurring from September to May.

MATERIALS AND METHODS

Four transects were laid out, each containing 12 traps, and each facing no more than one neighboring transect at a distance of ≥ 10 m (Fig. 1). Distances between traps were as follows: Transect 1: 4.0 m; Transect 2: 2.0 m; Transect 3: 1.0 m; Transect 4: 0.5 m.

Ethylene glycol was used as the collecting medium, in uncovered clear plastic cups (8.5 cm diameter) installed one week prior to the first trapping date to avoid a digging-in effect (Joosse and Kapteyn 1968). Traps were approached along the same pathway at all times, and were handled from a distance of ≥ 0.5 m.

At intervals of approximately 3 weeks, traps were activated and emptied on 5 consecutive days, i.e. August 3 through 7 and 26 through 30; September

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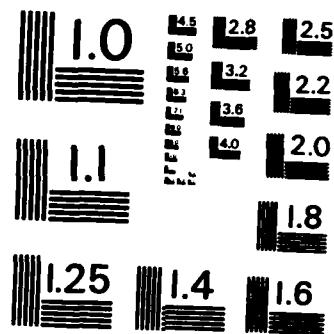
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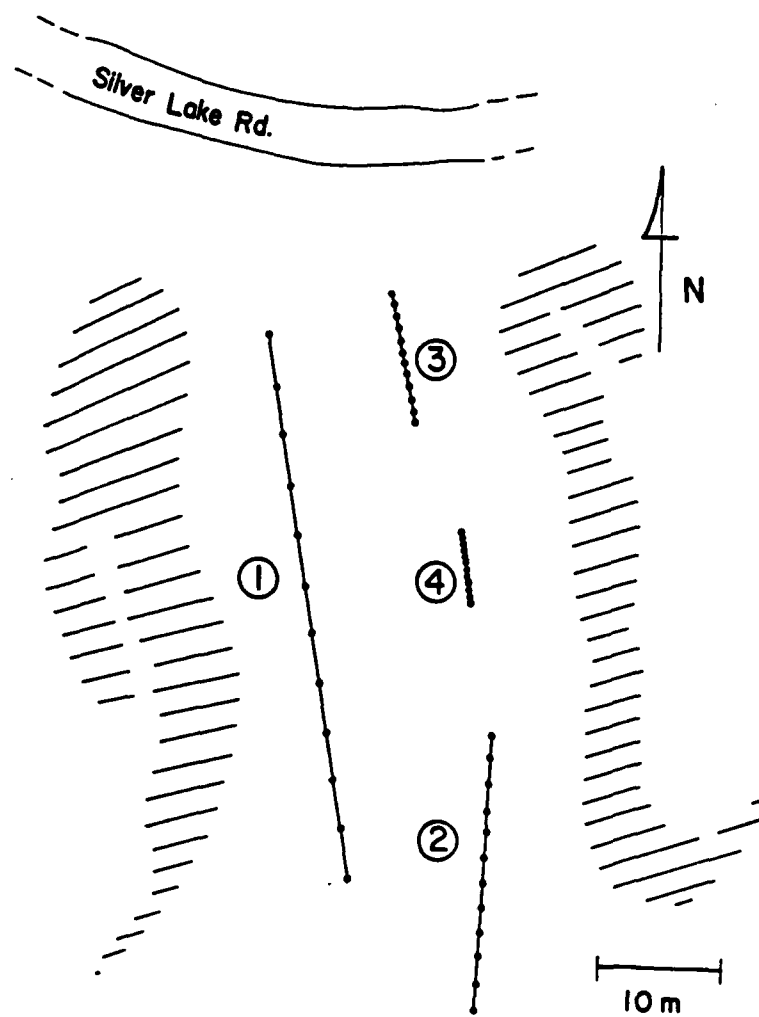


Fig. 1. Disposition of trapping transects in the Silver Lake site.

13 through 17; and October 4 through 8. Twenty samples (4 periods x 5 days each) were thus obtained from each trap.

RESULTS

1. Total arthropods and abundant taxa

Winged Diptera, Hymenoptera and Lepidoptera were excluded from totals discussed below. Hypopi (mainly of Acaridae) tended to outnumber all other mites combined, especially in traps with larger arthropods; they were also excluded because they did not actively enter traps.

The first sampling period yielded the largest catches, probably due to seasonally high active densities (Table 1). If trap distance had affected capture rates, ranking of transects should have been possible (i.e., 1 through 4 based on increasing or decreasing numbers caught). However, mean catches were essentially equal in 1 and 3, and in 2 and 4; and seasonal changes in numbers, minor after the first period (Table 1), were parallel in all transects.

Table 1. Mean \pm SE arthropods caught per trap, using total 5-day catch per trap (n= 12 traps per transect). Winged Diptera, Hymenoptera and Lepidoptera, and hypopi, excluded.

	TRANSECT			
	1 (4.0 m)	2 (2.0 m)	3 (1.0 m)	4 (0.5 m)
Aug 3-7	89.0 \pm 13.3	64.1 \pm 5.3	80.6 \pm 5.3	69.1 \pm 6.0
Aug 26-30	35.1 \pm 3.4	23.6 \pm 2.8	35.3 \pm 5.5	22.7 \pm 2.3
Sep 13-17	36.2 \pm 4.3	32.7 \pm 3.3	36.2 \pm 3.1	32.0 \pm 3.8
Oct 4-8	30.8 \pm 3.8	28.2 \pm 2.4	32.4 \pm 1.9	27.6 \pm 2.0

Among the six most frequently captured orders, Collembola, Acarina and

Coleoptera were prevalent (Fig. 2). Mites and Collembola, both more abundant in transects 1 and 3, seemed responsible for the larger catches in those transects (Table 1). Diplopoda (Fig. 2) consisted mainly of Uroblaniulus canadensis (Newport), with distinctly stage-specific activity (86% adult and subadult). Spiders (Fig. 2) were predominantly unidentifiable immatures. Of 16 families total, four contributed almost equally to total catches: Lycosidae (21%), Micryphantidae (22%), Linvnhidae (21%) and Agelenidae (20%). Activity of adults was distinctly seasonal in some species: Bathvnhantes pallida (Ranks) and Centromerus persoluta (O.-P.-Cambridge) disappeared entirely after August 30 ; Centromerus sylvaticus (Blackwell) and Wadotes calcaratus (Keyserling) were trapped exclusively in October; on the other hand, the common lycosids Pirata marxi Stone and P. maculatus Emerton were active throughout the study period.

If tran-distances had taxon-specific effects, then only Opiliones seemed to be affected, i.e. captured more efficiently by traps spaced 4.0 m apart (Fig. 2). However, catches of other taxa were transect-related in a non-linear way: Collembola totals, for instance, maintained a constant proportionality between the four transects, i.e. 3 > 1 > 4 > 2 through all periods (Fig. 2). This suggested that catches were proportional to different arthropod densities in different parts of the site.

In order to assess distributional (transect location) factors, three groups were further analyzed at the species level: Collembola (high species diversity, apparently transect-dependent numbers); Carabidae (fewer species, no apparent transect-catch relations); and Opiliones (least diverse, catches potentially affected by trap spacing).

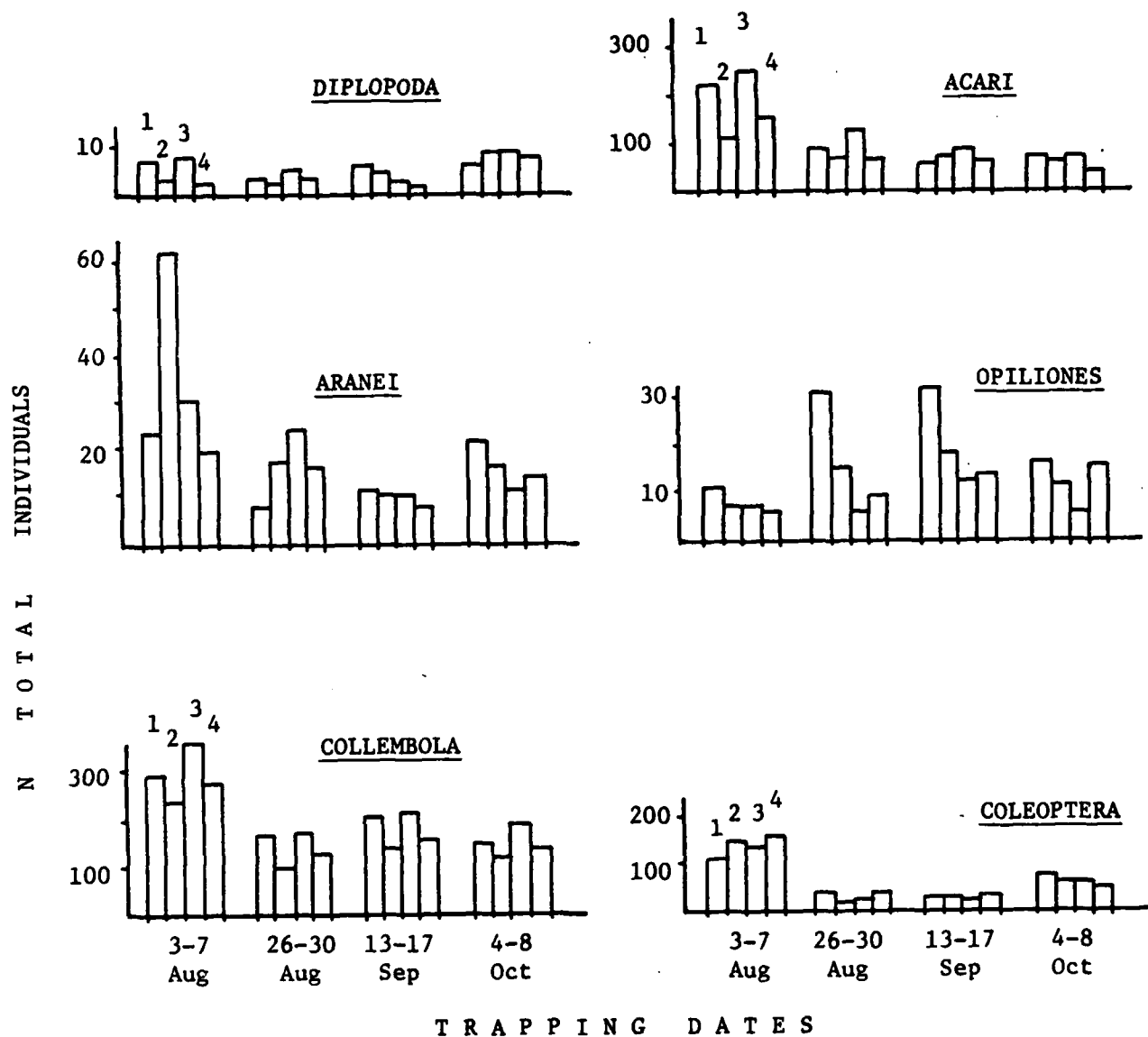


Fig. 2. Total catches per transect and period (summed over five days) of the most frequently trapped orders; numbers above bars = transect numbers (in the same sequence in each set of bars).

2. Collembola

List of species collected:

Hypogastruridae:

Odontella substriata Wrav
Xenylla acauda Gisin
Neanura muscorum (Templeton)
Pseudachorutes spp. complex

Isotomidae:

Isotoma (Desoria) nigrifrons Folsom
I. (Isotoma) viridis Bourlet

Entomobryidae:

Tomocerus (Pogonognathellus) flavescens Tullberg
T. (Tomocerina) lamelliferus Mills
Orchesella hexfasciata Harvey
Lepidocyrtus helenae Snider
L. hirtus Christiansen and Bellinger
L. lignorum (Fabricius)
L. paradoxus Uzel
L. violaceus Fourcroy
Entomobrya (Entomobryoides) purpurascens (Packard)
E. assuta Folsom
E. nivalis (Linne)
E. clitellaria Guthrie

Sminthuridae:

Sminthurides lepus Mills
Sminthurus butcheri Snider
Sminthurinus henshawi (Folsom)
S. conchyliatus Snider
S. intermedius Snider
S. quadrimaculatus (Ryder)
Dicyrtoma (Ptenothrix) marmorata (Packard)
Bourletiella (Bourletiella) hortensis (Fitch)
Arrhopalites amarus Christiansen
A. benitus (Folsom)

Most individuals belonged to the families Sminthuridae and Entomobryidae, each dominated by one species (S. henshawi and T. flavescens respectively) (Table 2). In each transect, the same two species furnished approximately 80% of each family total, the remainder occurring in very low numbers or singly.

Transect 4 yielded the highest number of species (Table 2), but unidentifiable immatures make diversity comparisons inconclusive. All transects had a number of Entomobrya and Lepidocyrtus spp. unique to them:

again, immatures of both genera also were captured in all transects. The six species listed in Table 2 together furnished 80% of the grand totals captured in each transect.

Table 2. Total number of each family trapped over the study period, and percent dominance for the prevalent species within each ($N_i/NT \times 100$).

TAXON	TRANSECT			
	1	2	3	4
N Sminthuridae	500	391	415	356
% <u>S. henshawi</u>	63.4	71.3	77.3	67.7
% <u>S. lepus</u>	24.6	16.4	9.4	11.5
N Entomobryidae	268	167	369	288
% <u>T. flavescens</u>	67.1	66.4	66.9	71.9
% <u>O. hexfasciata</u>	16.4	13.2	17.6	14.2
N Isotomidae	50	36	84	67
% <u>I. nigrifrons</u>	50.0	55.6	57.1	76.1
% <u>I. viridis</u>	50.0	44.4	42.9	23.9
N Hypogastruridae	37	15	70	11
TOTAL N SPECIES	17	17	20	21

Overall, sminthurid active density increased as the season progressed. S. henshawi determined this trend, counteracting that of all other species (Fig. 3). Entomobryid activity decreased (Fig. 4). Isotoma nigrifrons was particularly active in September (Fig. 5), coincident with its marked vertical migration from litter into the soil (unpub. data).

The proportionality discussed earlier (numbers in transect 3 > 1 > 4 > 2) was repeated only by T. flavescens, and only on three of four dates (Fig. 4). Whether a transect (location) effect existed was assessed by testing effects of season, i.e. 4 periods, and effects of transects for their independence (chi-square approximation). Lack of independence was significant for catches of S. henshawi ($P < .001$) and T. flavescens ($P < .005$), and for total catches of Sminthuridae ($P < .025$) and Entomobryidae ($P < .005$).

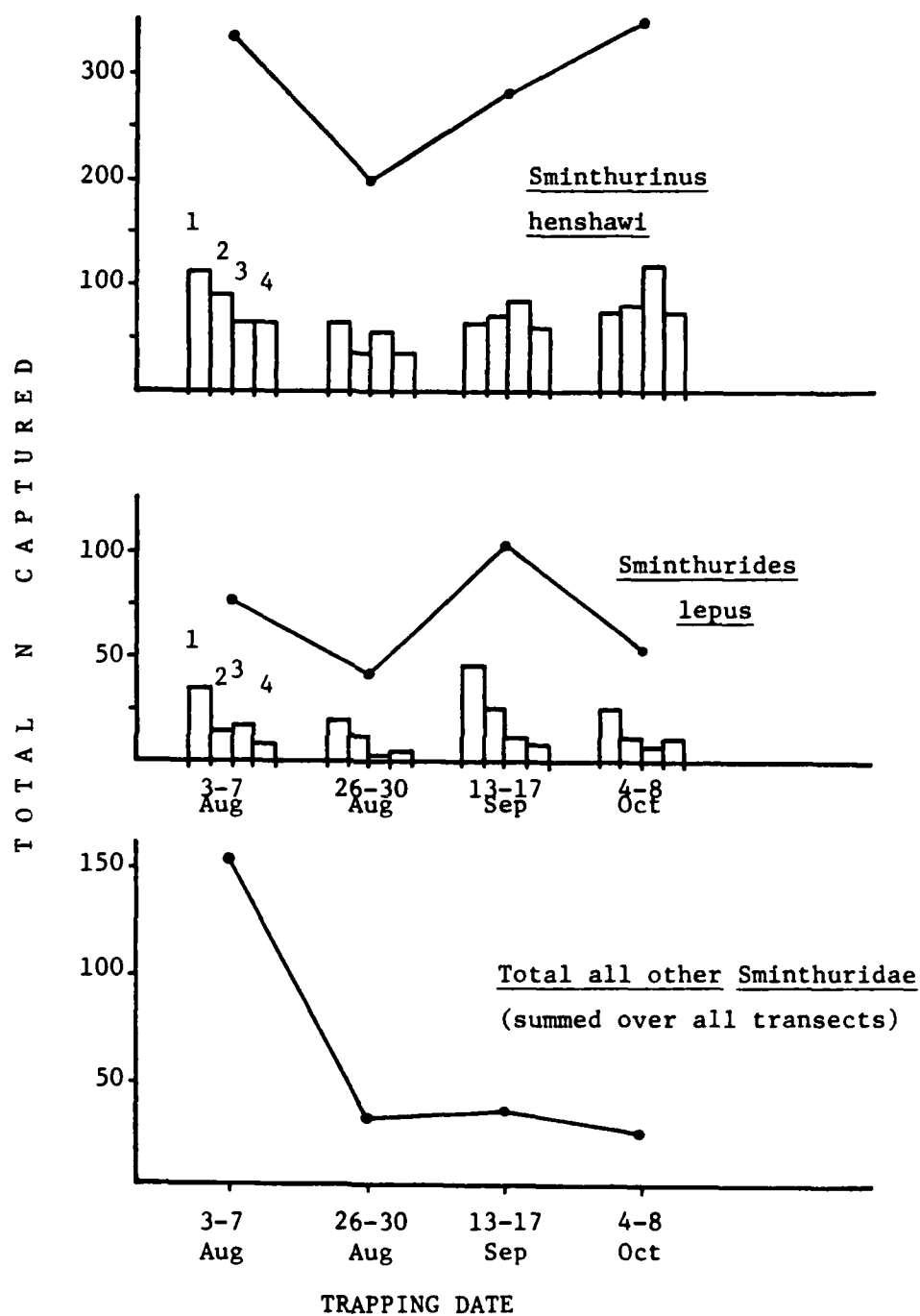


Fig. 3. Total number of selected sminthurids captured per transect and period (bars, transects 1 through 4), and overall totals per trapping period (solid line, sum of all transects).

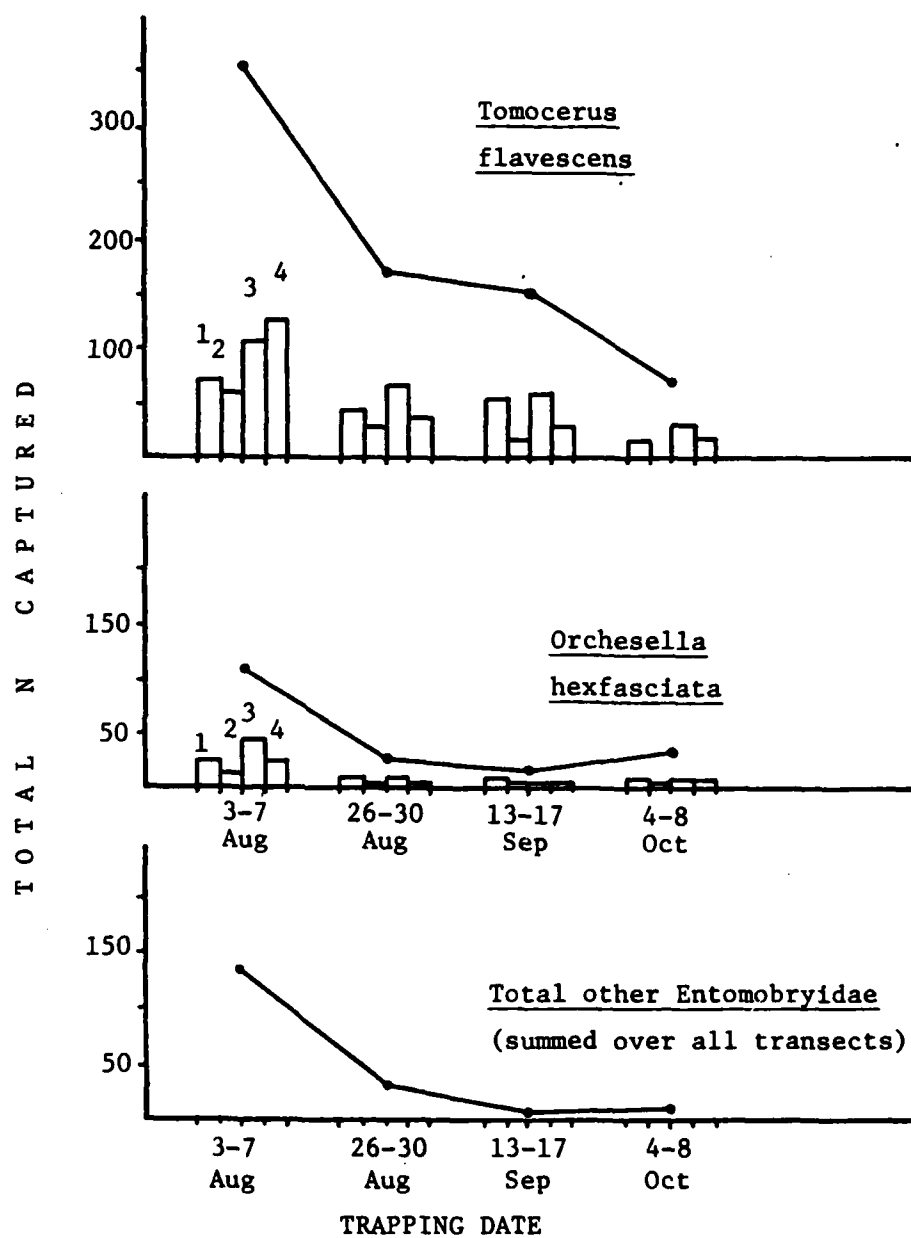


Fig. 4. Catches of selected entomobryids per transect within each period (bars, transects 1 through 4) and overall totals per period (solid line, sum of all transects).

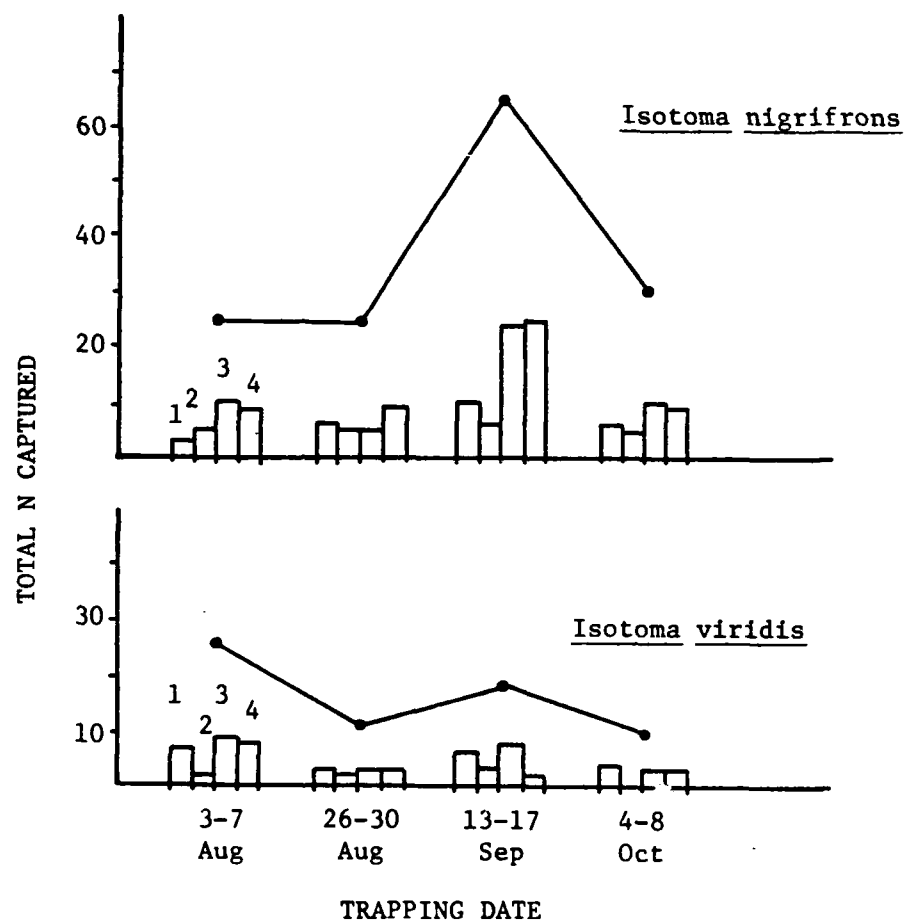


Fig. 5. Catches of isotomids per transect within each period (bars), and totals per period (solid line, sum of all transects).

3. Carabidae

List of species captured:

Pterostichus melanarius Illiger
P. pensylvanicus LeConte
P. coracinus Newman
P. novus Straneo
P. adoxus Say
P. adstrictus Eschscholtz
Synuchus impunctatus Say
Calathus ingratus Dejean
C. gregarius Say
Cymindis cribricollis Dejean
Carabus sylvosus Say
Notiophilus aenus Herbst

Carabidae constituted 62 to 87% of all Coleoptera captured per period. Total numbers per transect, summed over all dates, were clearly equal, four species together furnishing approximately 90% of the site's carabid fauna in summer and fall (Table 4). High numbers of P. melanarius and S. impunctatus in early August (Fig. 6) reflected the end of their summer activity period (Lindroth 1969; Barlow 1970); the October activity peak of P. pensylvanicus (Fig. 6) represented the second of the season, mainly due to appearance of teneral adults (Barlow 1970; Nesmith 1985).

Table 4. Total carabids caught over the study period, and percent occurrence of the four most common species.

	TRANSECT			
	1	2	3	4
N total Carabidae	167	164	150	165
N total species	9	9	11	9
% <u>P. melanarius</u>	25.1	40.2	22.0	42.4
% <u>P. pensylvanicus</u>	30.5	26.8	34.0	16.4
% <u>S. impunctatus</u>	22.2	15.9	20.7	17.6
% <u>P. coracinus</u>	10.8	8.5	9.3	11.5
Totals, %	88.6	91.4	86.0	87.9

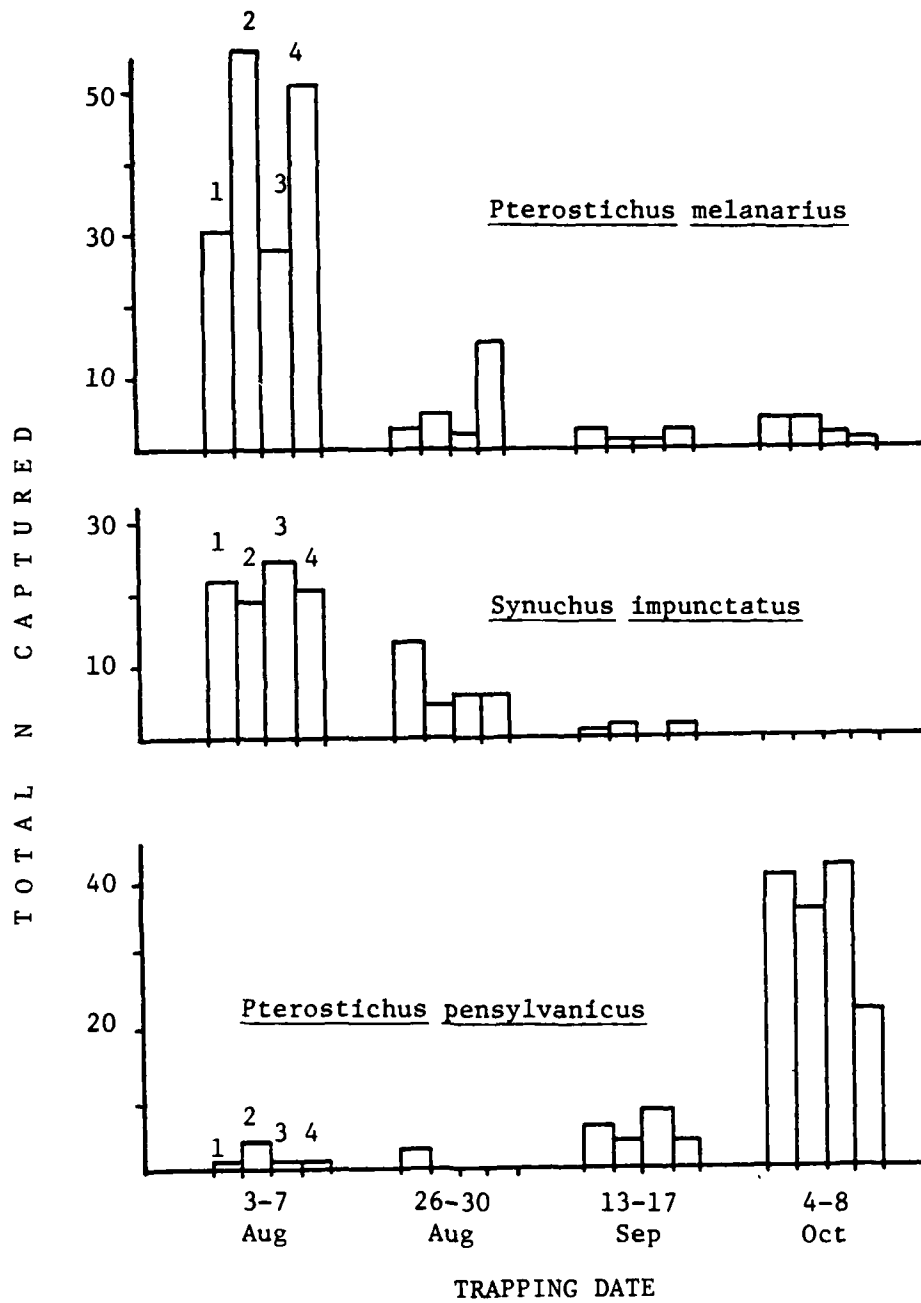


Fig. 6. Transect-specific catches of three common species of carabids in each trapping period.

Traps in the eastern part of the site (transects 2 and 4) caught relatively more P. melanarius and fewer P. pensylvanicus than 1 and 3 (Table 4). Tests of independence (season and transect effects), however, gave results which were not significant for three of the common species, and only marginally so for P. melanarius ($P < .1$).

4. Opiliones

Species captured:

Phalangidae

Caddo boopis Crosby

Odiellus pictus (Wood)

Leiobunum nigripes (Weed)

L. politum Weed (rare, transects 1, 2, 4)

Ischyropsalidae

Sabacon crassipalpi (L. Koch)

Nemastomatidae

Crosbycus dasycnemus (Crosby) (rare, transects 1, 4)

Both C. boopis and O. pictus showed declining activity in the fall, while S. crassipalpi became increasingly active in September and October, and L. nigripes activity peaked in mid-September (Fig. 7). A high active density of L. nigripes was probably associated with maturation to adulthood: frequency of immatures, in percent of total catch, progressively declined from 60% in early August to 48% in late August, 8% in September and 4% in October.

Catches were transect- (location-)specific: transect 4 traps caught more S. crassipalpi than any other traps, while transect 1 traps yielded most individuals of L. nigripes (Fig. 7). Since L. nigripes was numerically dominant (56% of all specimens), transect 1 seemed superior to all others for catching opiliones as a group (Fig. 2). Lack of independence (season/transect effects) could be shown only marginally for S. crassipalpi

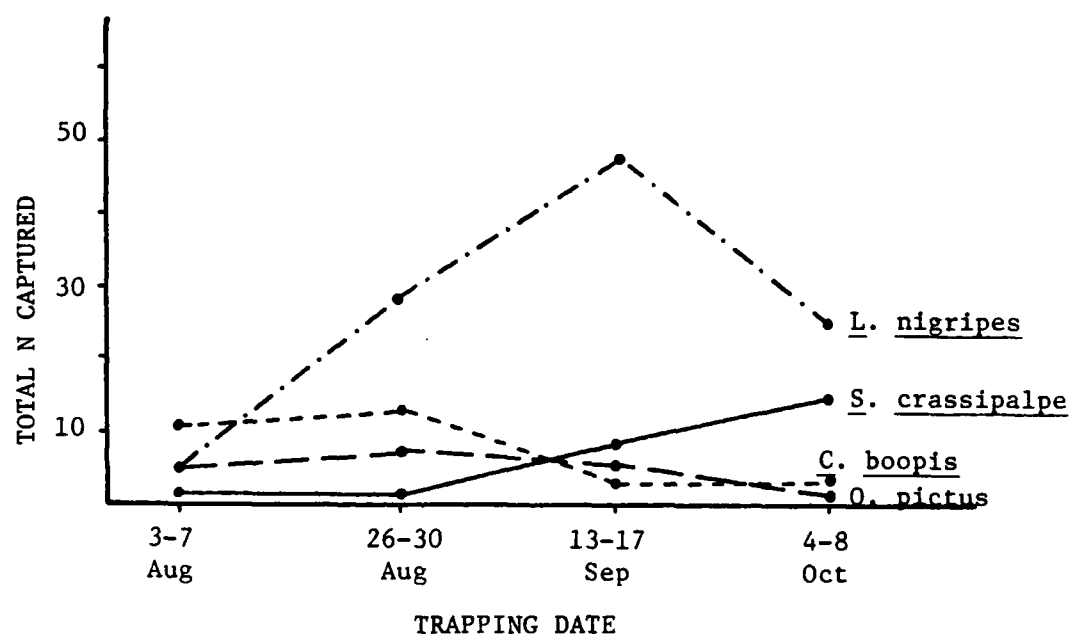


Fig. 7. Total catches (summed over transects) of four species of opilionids during each trapping period.

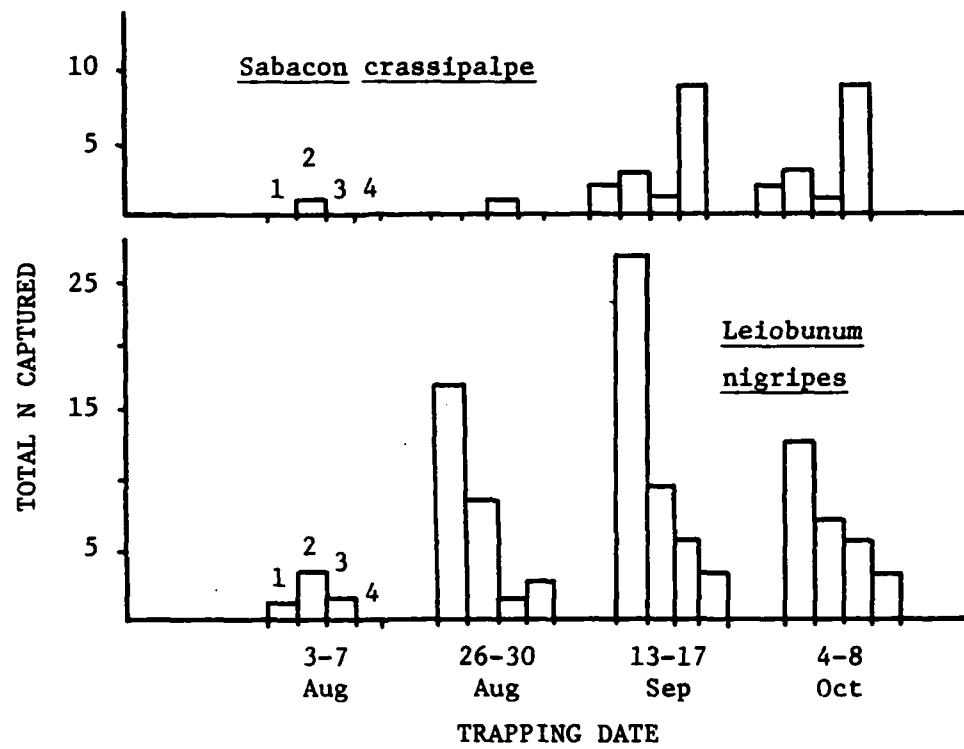


Fig. 8. Transect-specific catches of S. crassipalpe and L. nigripes during each trapping period.

as well as L. nigripes ($P < .1$), probably due to frequent low catches.

DISCUSSION

In other studies, disposition of traps relative to each other varies from random (e.g., Benest and Cancela da Fonseca 1980) to a number of different patterns such as concentric circles (Carter 1980) or grids (Dennison and Hodkinson 1984). In non-random designs, distance between traps is generally held constant within any one habitat. In the present study, transect distribution over the site essentially resulted in one line of 12 traps versus three lines of 36 traps total (Fig. 1). Removal of arthropods was thus three times more pronounced along the eastern side of the site. Conceivably, depletion of populations could have been compounded by closely spaced traps, at least for smaller, less mobile species. No such effects materialized, possibly because all transects were equally open to immigration on one side, and the study period was relatively short.

The data showed, however, a transect (location) effect: assuming that activity patterns of the species investigated did not differ over a distance of 30 or 40 m, then catches reflected transect-specific density variations of several populations. Indeed, had a trap-distance effect existed, these variations over different parts of the site would not have become apparent.

Unexpectedly, the results thus indicated that trapping can be a valid means of comparing faunal densities in relative terms. By extrapolation (from two parts of a site to two different sites), trapping could be used to compare the faunas of two sites, as long as their habitat and climate characteristics are closely similar. Ericson (1979), working with a more extensive set of data on field carabids, came to similar conclusions.

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APPENDIX C

MANUSCRIPT 2

(Pedobiologia, in press)

Techniques for sampling earthworms and cocoons from leaf litter, humus and soil¹⁾.

by

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and

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¹⁾ Research supported by Department of the Navy Contract No. N00039-81-C-0357.

1. Introduction

While preparing for a long-term ecological study in Michigan forests, we needed to develop a sampling technique which would yield accurate estimates of all lumbricid life stages in leaf litter, humus, and soil. Existing techniques fall into two basic categories: handsorting, and extraction by chemical irritants such as formalin (Raw 1959). Chemical extraction recovers only active earthworms, resulting in seasonally variable sampling efficiencies (Bouche 1969) and potentially inaccurate population estimates. Handsorting, on the other hand, recovers earthworms regardless of their state of activity, and can yield 95% of total worm biomass and 80% of total numbers (Axelsson et al 1971). However, small dark individuals are often overlooked (Raw 1960). Cocoons, typically encapsulated in soil, are generally not found at all: neither Satchell's (1969) review, nor more recent publications by Terhivuo (1982) and Springett (1981) take sampling of cocoons into consideration.

Some investigators improved recovery rates for small worms either by repeated handsorting (Axelsson et al 1971) or by wet-sieving of previously handsorted soil, a modification which promised to be adaptable to cocoon sampling (Raw 1960). Indeed, Gerard (1967) estimated cocoon densities by wet-sieving, but did not quantify the efficiency of his technique.

We combined the most successful methods for sampling all lumbricid life stages, and thus developed a two-phase procedure: handsorting followed by wet-sieving. The procedure could be applied to both humus and soil samples, but proved tedious and unreliable for estimating worm populations in leaf litter. A separate method, formalin extraction, was therefore optimized for use on litter

samples. Descriptions of both protocols, and results of their validation, are given below.

2. Methods

Samples were taken in two maple-basswood forests in Dickinson Co., Michigan, with sandy loam soils and well-developed mull humus layers, but different lumbricid populations. By sampling both sites, a total of four species were obtained: two epiges, Dendrobaena octaedra (Savigny) and Lumbricus rubellus (Hoffmeister); and two endoges, Octolasion tyrtaeum (Savigny) and Aporrectodea tuberculata (Eisen). Sampling was accomplished by running a knife along the inside periphery of a 25 x 25 cm frame so that a 1/16 m² sample of litter could be removed and bagged. The dark humus layer and two underlying 10 cm soil increments were then excavated and placed in large plastic bags.

Humus and soil samples were subjected to a sorting/sieving process, diagrammatically shown in Figure 1. The first step was conventional handsorting (step A), followed by two consecutive washes through a coarse and a fine screen. The first (5.0 mm mesh) retained stones, roots, and debris, which were turned, rinsed, and searched several times (step B). All material passing through the coarse sieve was caught in a large tray, dumped onto a second, fine sieve (1.5 mm mesh), and rinsed repeatedly to eliminate soil particles (step C). What remained of the sample consisted of worm casts, small pebbles, organic debris, worms and cocoons. During subsequent washes, soil capsules hiding cocoons were broken by gentle manual pressure. Each wash was followed by a thorough search, until the sample was reduced to a thin layer of debris incapable of obscuring specimens (step C).

Humus (n = 32 samples), which harbored the largest number of worms and cocoons, was used to assess the reliability of the method. Two potential sources of error were addressed. First, worms and cocoons present on the fine sieve could simply be overlooked. At the end of step C, samples were therefore rinsed repeatedly until two consecutive searches brought no further results (step D). Secondly, active worms and small cocoons could have passed through the fine sieve. This was tested by reserving the residues of every wash performed in steps C and D, rinsing them through a 1.0 mm net, and carefully searching the materials caught on the net (step E).

A modified chemical expellent technique, formalin extraction, was used on litter samples. They were sprinkled with enough water so that a small amount collected at the bottom of the bags, thereby ensuring that worms could become fully active. After 24 hours, each sample was placed in a wire basket fashioned from 10.0 mm mesh screening and resting approximately 5 cm above the bottom of an eight-liter bucket. Dilute formalin (0.025%) was added to cover the sample, driving worms from the litter to the bottom of the bucket. One hour later, the basket was lifted out and the solution was poured through a 1.0 mm mesh sieve, which was held against a white background so that worms could easily be collected from it.

In order to assess the method's efficiency, 16 litter samples were thoroughly dried to kill any worms they contained, then were re-wetted. Forty worms (immatures and adults of D. octaedra and L. rubellus) were added to each sample, and formalin extraction was performed 24 hours later.

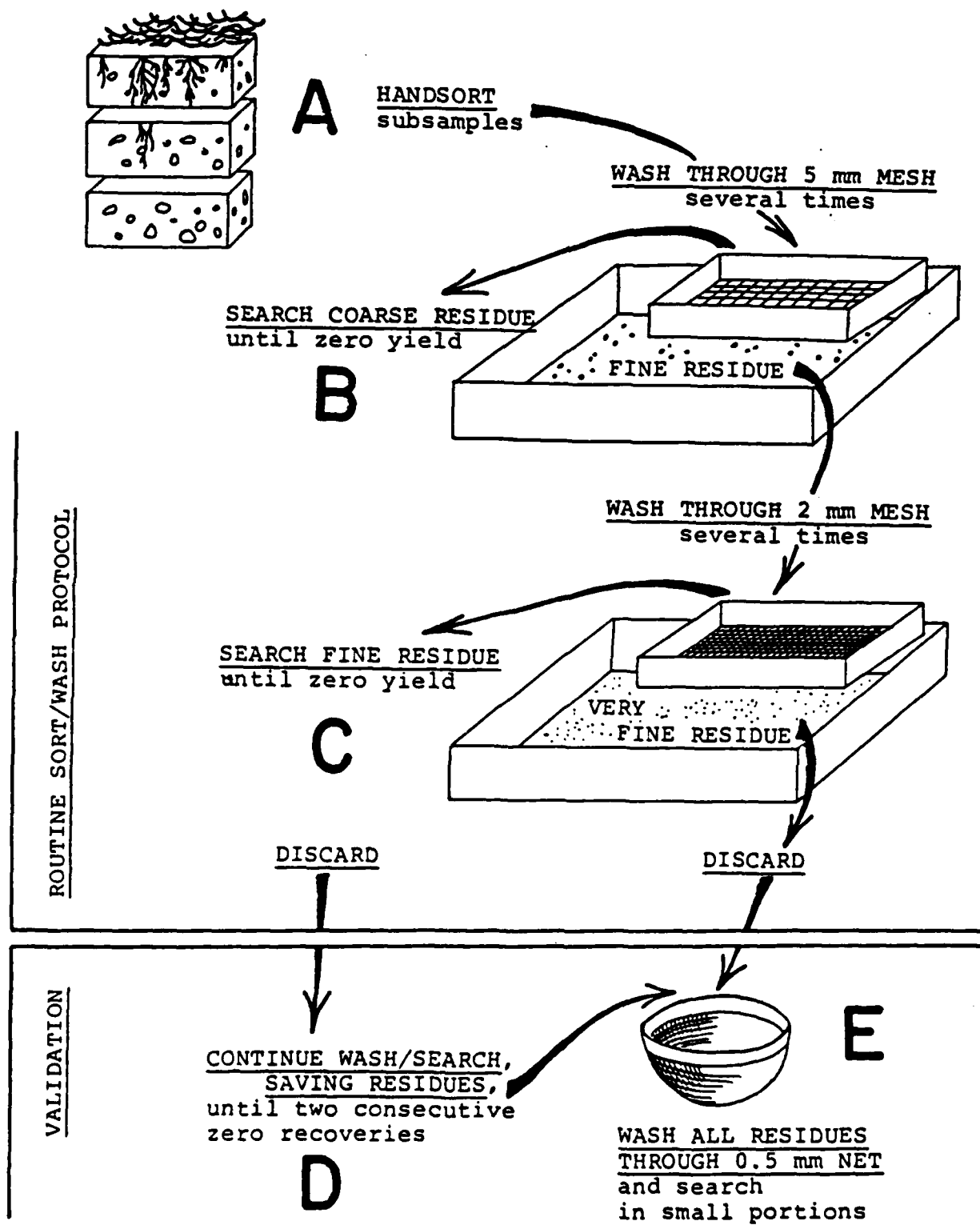


Figure 1. Diagram of consecutive steps used in the routine sort/wash protocol and its validation.

3. Results and Discussion

Formalin extraction of worms from leaf litter gave satisfactory results. Aside from the hour-long extraction period per se, processing of each sample required approximately 15 minutes. Based on 16 validated samples, the technique yielded an average of 96.0 ± 1.0 (SE) percent of the introduced worms. It was thus a rapid and reliable method for obtaining estimates of litter-inhabiting lumbricid populations.

The efficiency of sorting/sieving was high for all species (Tables 1 and 2). Percentages given in these tables are based on the assumption that all steps combined (A through E) recovered 100% of the worms and cocoons in a sample. In the routine procedure (steps A-C) 2138 specimens were found, while combined validation steps D and E yielded 55. We believe that any processing beyond these many consecutive washes and searches would have revealed very few additional worms and cocoons.

By handsorting alone, more than 90% of immatures of all species were found and, except for the small-bodied D. octaedra (Table 1), 100% of adults. Summed over all species and sizes, our efficiency of 88% compared well with other studies. Axelsson et al (1971), by handsorting once, obtained 86% of immatures, and Nelson and Satchell (1962) reported efficiencies ranging from 74 to 100% for various earthworm species.

Cocoon recovery by handsorting was generally poor and was affected by their physical characteristics. The smooth, white cocoons of D. octaedra were found at the highest rates (14.4%, Table 1). Those of L. rubellus were usually missed (Table 1), because they were dark, rough-textured, and tightly encapsulated with

Table 1. Validation of sorting/sieving efficiency for two epigeic species: percent of total numbers and total weights (in parentheses) recovered in successive steps (for key, refer to Fig. 1).

Process	Species and Stage					
	<u>Dendrobaena octaedra</u>			<u>Lumbricus rubellus</u>		
	<u>Cocoons</u>	<u>Immatures</u>	<u>Adults</u>	<u>Cocoons</u>	<u>Immatures</u>	<u>Adults</u>
(A)	14.4	90.5 (96.4)	98.6 (97.0)	1.6	90.0 (96.4)	100.0 (100.0)
(B+C)	84.1	6.3 (2.4)	1.4 (3.0)	84.1	7.6 (3.2)	0.0 (0.0)
(D)	1.5	1.0 (1.0)	0.0 (0.0)	12.8	1.5 (0.2)	0.0 (0.0)
(E)	0.0	2.2 (0.2)	0.0 (0.0)	0.0	1.5 (0.2)	0.0 (0.0)
missed (D+E)	1.5	3.2 (1.2)	0.0 (0.0)	14.3	3.0 (0.4)	0.0 (0.0)
recovered (A+B+C)	98.5	96.8 (98.8)	100.0 (100.0)	85.7	97.0 (99.6)	100.0 (100.0)
Total n (A+B+C+D+E)	659	411	73	188	66	9

Table 2. Validation of sorting/sieving efficiency for two endoge species, in percent of total numbers and total weights (in parentheses) (for key, refer to Fig. 1).

Process	Species and Stage					
	Octolasion tyrtaeum			Aporrectodea tuberculata		
	Cocoons	Immatures	Adults	Cocoons	Immatures	Adults
(A)	11.7	90.5 (97.6)	100.0 (100.0)	12.5	90.1 (97.4)	100.0 (100.0)
(B+C)	86.9	6.9 (1.6)	0.0 (0.0)	81.2	9.9 (2.4)	0.0 (0.0)
(D)	1.2	2.5 (0.7)	0.0 (0.0)	6.3	0.0 (0.0)	0.0 (0.0)
(E)	0.2	0.6 (0.1)	0.0 (0.0)	0.0	0.0 (0.0)	0.0 (0.0)
missed (D+E)	1.4	3.1 (1.2)	0.0 (0.0)	6.3	0.0 (0.4)	0.0 (0.0)
recovered (A+B+C)	98.6	96.9 (99.2)	100.0 (100.0)	93.7	100.0 (100.0)	100.0 (100.0)
Total n (A+B+C+D+E)	486	158	58	16	71	8

soil.

Wet-sieving (steps B and C) was unnecessary for obtaining adults, but did yield 7.7% of total immatures of all species (Tables 1-2). Still, an average 2.3% of these individuals were missed, as a result of both sources of error postulated earlier. Thus, 2.2% of small D. octaedra were caught in validation step E (Table 1), indicating that they had escaped through the 1.5 mm screen into the normally discarded residue. By contrast, 2.5% of O. tyrtaeum immatures were recovered by validation step D (Table 2), proving that they had been overlooked during the routine searches of step C.

High cocoon yields proved to be the technique's greatest asset, since cocoons constituted 61% of total numbers and 8.9% of total lumbricid biomass. Again, those of L. rubellus had the highest chance of being missed (Table 1). Recovery exceeded 90% for A. tuberculata, (Table 2), and came close to 99% for D. octaedra (Table 1) and O. tyrtaeum (Table 2). A 1.5 mm mesh size was clearly appropriate for retaining even the smallest cocoons: all of those missed were recovered by validation step D (repeated rinsing and search of sample remains on the fine screen), not step E (washing of residues which had passed through the fine screen). A single O. tyrtaeum cocoon found on the 1.0 mm net (0.2% of total, Table 2) had probably been spilled out of the 1.5 mm sieve during washing.

Summarized over all species, sorting/sieving gives excellent results, allowing recovery of 97.7% of total immatures and adults, 96.7% of all cocoons, and 99.9% of non-cocoon lumbricid biomass. The entire procedure requires an average of 90 minutes per sample, its two phases possessing the following merits: handsorting recovers the bulk of immatures and adults, and is a step which cannot be omitted because it yields animals in good condition (wet-sieving damages large

worms). Wet-sieving as a second phase acts as a check on handsorting efficiencies, which vary from person to person, and allows reliable estimates of cocoon densities.

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Synopsis: Original scientific paper

Walther, P.B. and R.M. Snider. Techniques for sampling earthworms and cocoons from leaf litter, humus and soil.

Litter-inhabiting lumbricids were extracted by one-hour immersion in a dilute (0.025%) formalin solution. The method's efficiency, validated with 16 litter samples and 640 introduced worms, was 96%.

A technique for obtaining lumbricids and cocoons from humus and soil was developed. Samples were handsorted, then washed through a coarse and a fine screen. By handsorting, adults and most immatures were found. Repeated washing and search of sample remains proved to be highly reliable for recovering cocoons and small worms. Overall, sorting/sieving yielded 97.7% of all worms, 96.7% of cocoons, and almost 100% of worm biomass.

Key Words: Earthworms, sampling, cocoons, handsorting, formalin.

APPENDIX D

MANUSCRIPT 3

(submitted to Entomological News)

Larvae of Pterodontia flavipes Gray (Diptera:Acroceridae) occurring in
Podothrombium (Acari:Trombidiidae) and Abrolophus (Acari:Erythraeidae)¹

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¹Support for this research was provided by the Naval Electronic Systems
 Command through a subcontract to IIT Research Institute under contract
 N00039-81-C-0357.

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ABSTRACT: Larvae of Pterodontia flavipes Gray (Diptera:Acroceridae) were found parasitizing the mites Podothrombium sp. (Trombidiidae) and Abrolophus s.l. (Erythraeidae).

The larvae of Pterodontia flavipes Gray (Fig. 1) are parasitoids that are thought to enter their hosts at the articulations of the legs (King 1916). While these larvae normally parasitize spiders of the Family Lycosidae, this note describes their presence in mites of the Cohort Parasitengona.

Specimens were collected in pitfall traps set in a northern hardwood forest located in Dickinson County, Michigan. The forest was dominated by sugar maple (Acer saccharum) with basswood (Tilia americana) as the subdominant. Leatherwood (Dirca palustris) was the most abundant understory shrub. The pitfall trapping season ran from late July 1982 until early November 1982 and all Parasitengona collected were cleared in lactic acid and examined for the presence of P. flavipes larvae.

Of the 27 mites examined, three were found to be parasitized by the first instar larva of P. flavipes (11%); 2 Podothrombium sp. (F. Trombidiidae) and 1 Abrolophus s. l. (F. Erythraeidae). All parasitized mites were collected in September. In all three cases, only a single larva was found and each was located posterior to the articulations of the palp and anterior to Coxa II (Fig. 2). Due to the size of the mite relative to that of P. flavipes, it is probable that the mite would have been killed

before the larva entered its second instar (pers. comm. E. I. Schlinger)

ACKNOWLEDGEMENTS: I would like to thank E. I. Schlinger for identification of P. flavipes and W. C. Welbourn for identification of the mites.

LITERATURE CITED: King, D. L. 1916. Observations on the life history of Pterodontia flavipes Gray (Diptera). Ann. Entomol. Soc. Am. 9:309-321.

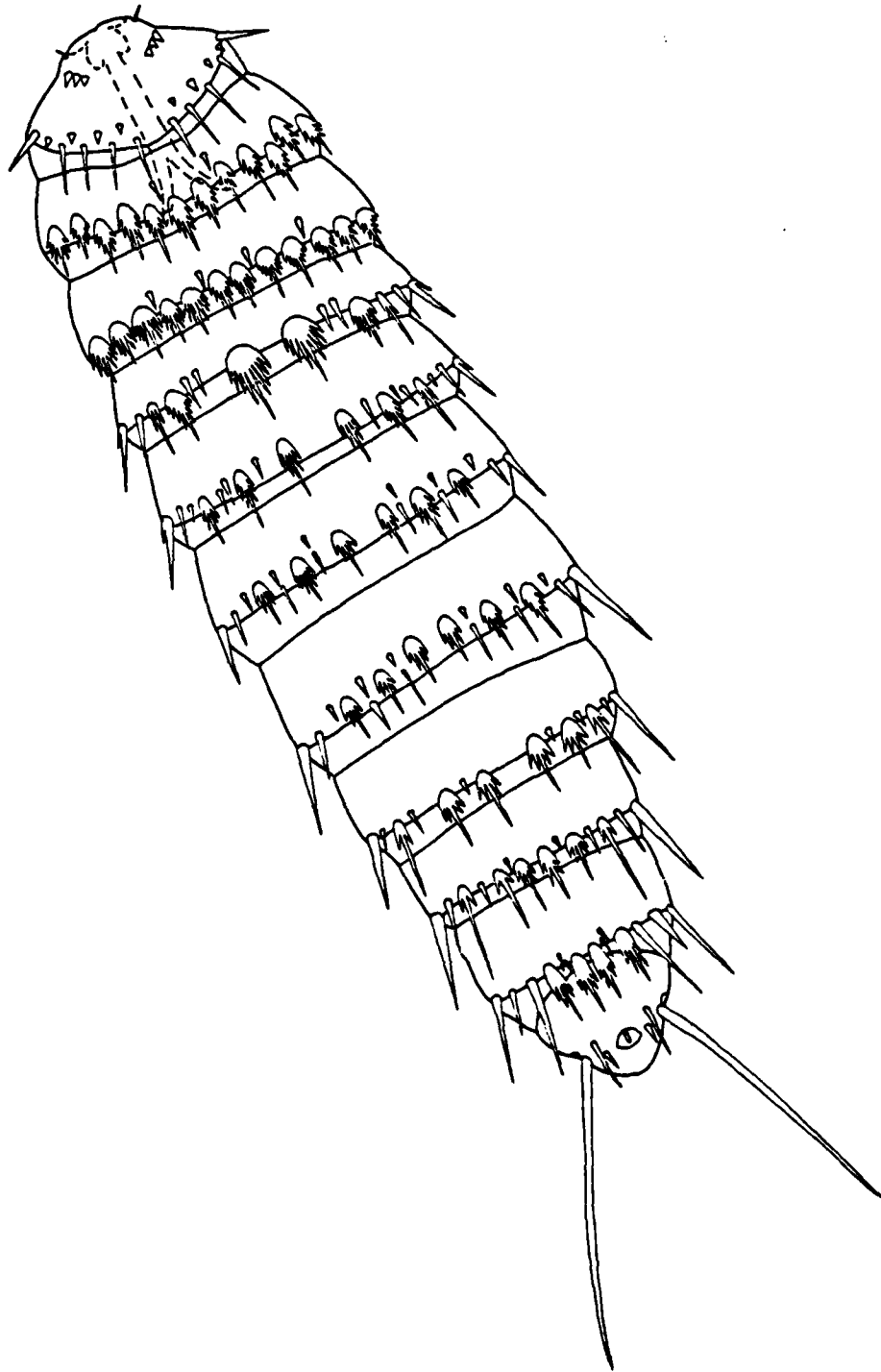


Fig. 1. First instar larva of Pterodontia flavipes Gray.

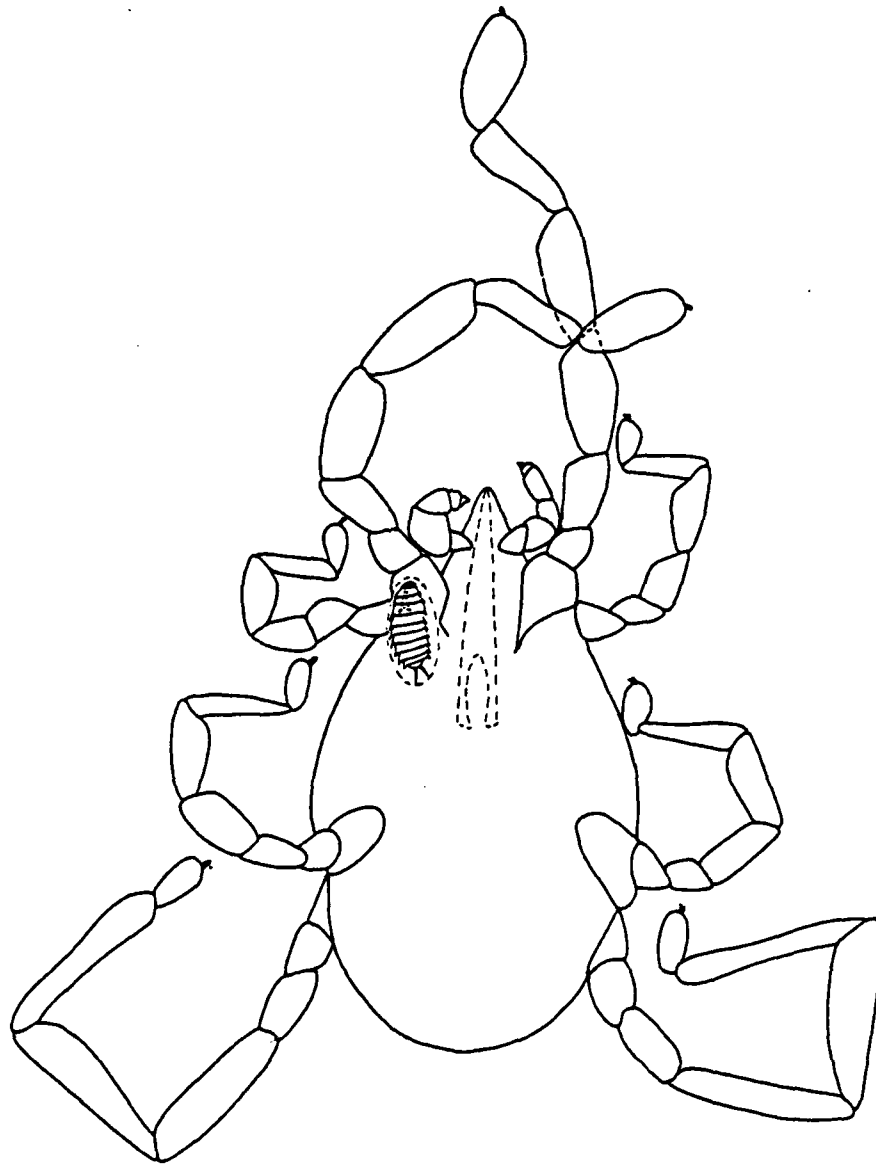


Fig. 2. Abrolophus s.l. showing the position of P. flavipes.

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